# Observations on the Pancreatic Islet Tissue of Young Diabetic Subjects

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Little is known of the succession of changes which take place in the pancreas of a young diabetic since direct observation is obviously impossible. Some idea of the alterations which occur may, however, be gained from study of the pancreases of diabetics dying at different stages of the disease. To this end we examined the pancreases of diabetics dying under the age of thirty years, and here summarize our findings and inferences.

## MATERIAL AND METHOD OF STUDY

All the available pancreatic sections of diabetic patients who were under thirty years of age at death were examined. Some were too autolyzed for quantitative examination. Five showing acute pancreatitis of varying severity were rejected because of the effect of a primarily exocrine inflammation upon the function of the islets. Two cases with hyalinization and fibrosis of the islets were rejected because of the difficulty in estimating the amount of functioning islet tissue remaining in partly hyalinized islets. Two cases were unacceptable because the date of onset of symptoms could not be ascertained. In the remaining forty-one cases the interval between the known onset of the disease and death varied from two days to nineteen years. In twenty-nine cases, sections from blocks of the head, body and tail of the pancreas were examined, and in twenty-seven of these cases the weight of the pancreas was known. In six cases two blocks were available and in a further six cases only one block was available for examination. The weight of the pancreas was known in only two of these twelve cases. The pancreases of twenty-two nondiabetic patients of similar age were used for comparison. Quantitative estimations by a method previously described[1] were made without knowledge of the age of the patient or of the duration of the disease.

The decision to use the six cases in which only a single block of the pancreas was available was made in light of previously unpublished observations. In a former investigation,[1] no constant variation in the distribution of islets in either the anteroposterior or vertical axes of the pancreas was noted. In contrast, the frequency distribution of islets in twelve transverse sections taken at equidistant intervals along the whole length of the pancreases of two diabetic and three nondiabetic subjects followed a fairly characteristic pattern in each pancreas (figure 1). It will be seen that sampling of a single block taken from the neck or the tail of the pancreas might yield unduly low or high results respectively. These, however, are not the usual sites from which a single routine histologic block is taken. Examination of a single block taken from the body of a pancreas with normal exocrine tissue will probably give a fair repre-

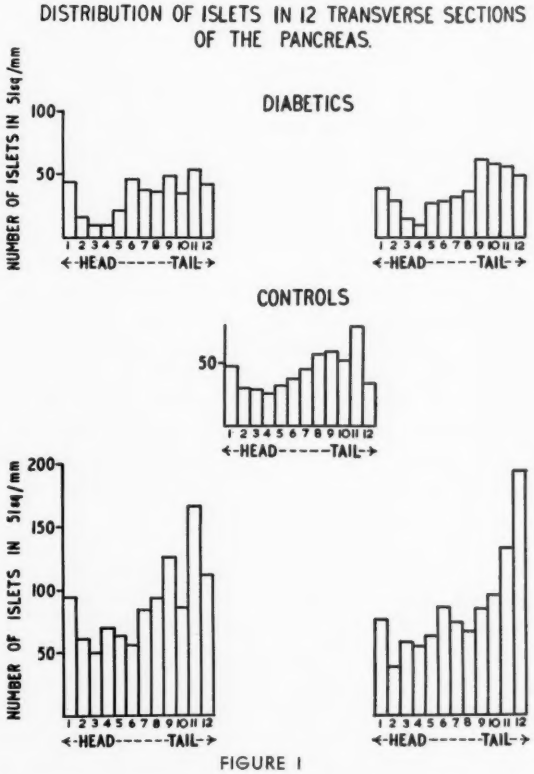


FIGURE 1

From the Departments of Pathology, The Western General Hospital, Edinburgh, Scotland, and the University of Edinburgh.

sentation of the proportion of islet tissue in the pancreas as a whole. Obviously this does not apply to pancreases which are fibrotic or grossly infiltrated by fat.

Measurement under low magnification of the size of the islets in twelve sections of the pancreases of each of three nondiabetic subjects suggested that there was no consistent variation in the size of islets in different parts of the pancreas. The validity of applying this observation to diabetic material was determined by estimating the area size of fifty islets at the magnification subsequently used in the investigation.[1] The results (table 1) suggested that the mean size of fifty randomly selected islets was fairly constant in all portions of the pancreas. For the purpose of the subsequent investigation[1] the mean size of one hundred islets distributed evenly between the sections available was estimated. Such an estimate performed on sections from different levels of a single block is unlikely to deviate significantly from the true mean islet size of the whole pancreas.

These considerations prompted us to use the six cases (cases 10, 19, 29, 37, 38, 41—tables 2 and 3) in which only one block was available. Analysis of the findings on completion of the investigation has shown that the results obtained from them were similar to those in which sections of the head, body and tail of the pancreas were examined, and that none of the trends exhibited or the conclusions drawn would be affected by their removal from the final results.

## RESULTS

The results are detailed in tables 2, 3 and 4. Eighteen patients died within eight weeks of the onset of diabetes and are grouped as "acute" cases. The remaining twenty-three died at intervals varying from nine months to nineteen years after the onset of diabetes and are termed "chronic."

*Weight of pancreas.* The weight of the pancreas was known in twenty-seven of the diabetic cases and in fifteen of the nondiabetic subjects who served as controls. Because of the wide scatter in the ages of the patients, estimation of the mean weights over the whole range serves no useful purpose. In patients of the age of fourteen years and over, the mean pancreatic weights were: fourteen chronic cases—38.3 gm.; five acute cases—51.6 gm.; ten control cases—63.9 gm. The differences between the chronic and acute diabetics, and between the chronic diabetics and controls are significant, but the difference between the acute diabetics and the controls does not attain statistical significance ($P>0.2$).

*Size of pancreatic islets.* The size of pancreatic islets was measured in terms of squares (1 square = 0.0024 sq. mm.). It varied quite widely and in each group showed a tendency to increase with age and with the age of onset of the disease (figures 2 and 3). In contrast it tended to diminish in proportion to the duration of the disease, slowly and gradually in the chronic diabetics, and more rapidly in the acute diabetics (figure 4).

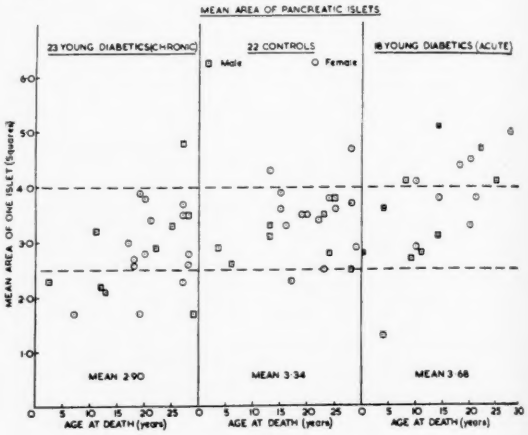


FIGURE 2

The mean size of the islets of the chronic diabetics (2.90) was significantly less than that of the acute diabetics (3.68) and the controls (3.34). The acute diabetic group and the controls were not strictly comparable since they contained three and one children respectively under the age of five. Over the age of five the mean size of the islets of the acute diabetics was significantly greater than that of the controls.

TABLE 1

Mean size of fifty islets (in squares) in each of twelve sections of the pancreases of four diabetic subjects

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Mean of twelve sections |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diabetic | 4.50 | 5.06 | 4.54 | 4.72 | 4.74 | 5.02 | 4.20 | 4.60 | 4.22 | 4.44 | 4.38 | 4.64 | $4.58 \pm 0.26$ |
| Diabetic | 2.92 | 2.80 | 2.88 | 2.88 | 3.08 | 3.14 | 2.64 | 2.86 | 2.88 | 2.92 | 2.78 | 2.90 | $2.89 \pm 0.13$ |
| Diabetic | 3.92 | 4.12 | 3.98 | 4.26 | 3.76 | 4.64 | 3.98 | 3.60 | 3.82 | 4.10 | 3.94 | 3.80 | $3.99 \pm 0.27$ |
| Diabetic | 1.98 | 1.74 | 2.00 | 2.16 | 1.78 | 1.92 | 1.72 | 1.90 | 2.00 | 1.96 | 1.94 | 1.98 | $1.92 \pm 0.12$ |

TABLE 2

Diabetic cases (chronic)

| Case no. | Sex | Age at death (years) | Duration of diabetes (years) | Age at onset | Weight of pancreas gm. | Percentage of islet tissue | Weight of islet tissue gm. | Mean area of one islet (squares) |
|---|---|---|---|---|---|---|---|---|
| 1 | M | 2½ | 9/12 | 1 9/12 | 8.4 | 1.74 | 0.15 | 2.3 |
| 2 | F | 7 | 6 | 1 5/12 | 26.1 | 0.59 | 0.15 | 1.7 |
| 3 | M | 11 | 5 | 6 | 28.4 | 1.46 | 0.41 | 3.2 |
| 4 | M | 12 | 2½ | 9½ | 40.0 | 0.33 | 0.13 | 2.2 |
| 5 | M | 13 | 3 | 10 | 26.1 | 1.18 | 0.31 | 2.1 |
| 6 | F | 17 | 2 | 15 | | 0.83 | | 3.0 |
| 7 | F | 18 | 4 | 14 | 46.8 | 0.44 | 0.22 | 2.7 |
| 8 | F | 18 | 2½ | 16 | | 0.54 | | 2.6 |
| 9 | F | 19 | 5 | 14 | 22.0 | 0.95 | 0.21 | 3.9 |
| 10 | F | 19 | 6 | 13 | | 0.15 | | 1.7 |
| 11 | F | 20 | 7 | 13 | 27.8 | 0.69 | 0.18 | 3.8 |
| 12 | F | 20 | 5 | 15 | 32.9 | 0.58 | 0.19 | 2.8 |
| 13 | F | 21 | 1 5/12 | 19 | 40.7 | 0.81 | 0.33 | 3.4 |
| 14 | M | 22 | 5 | 17 | 69.4 | 0.18 | 0.12 | 2.5 |
| 15 | M | 25 | 10 | 15 | 35.9 | 0.40 | 0.14 | 3.3 |
| 16 | F | 27 | 14 | 13 | 20.4 | 0.23 | 0.05 | 2.2 |
| 17 | F | 27 | 6 | 21 | 44.4 | 0.76 | 0.34 | 3.7 |
| 18 | M | 27 | 2½ | 24 | | 0.67 | | 4.8 |
| 19 | F | 27 | 3 | 24 | 14.0 | 1.04 | 0.14 | 3.5 |
| 20 | F | 28 | 14 | 14 | 47.2 | 0.32 | 0.15 | 2.6 |
| 21 | F | 28 | 19 | 9 | 21.2 | 0.42 | 0.08 | 2.6 |
| 22 | M | 28 | 6 | 22 | 43.6 | 0.86 | 0.38 | 3.5 |
| 23 | M | 29 | 12 | 17 | 70.0 | 0.33 | 0.26 | 1.7 |

TABLE 3

Diabetic cases (acute)

| Case no. | Sex | Age at death (years) | Duration of diabetes | Age at onset (years) | Weight of pancreas gm. | Percentage of islet tissue | Weight of islet tissue gm. | Mean area of one islet (squares) |
|---|---|---|---|---|---|---|---|---|
| 24 | M | 11/52 | 5 days | 11/52 | | 3.94 | | 2.8 |
| 25 | F | 3 11/12 | 6 weeks | 3 10/12 | | 1.50 | | 1.3 |
| 26 | M | 4 | 2 weeks | 4 | 12.4 | 2.86 | 0.37 | 3.6 |
| 27 | M | 8 | 12 days | 8 | | 2.71 | | 4.1 |
| 28 | M | 9 | 6 weeks | 9 | 17.7 | 1.15 | 0.20 | 2.7 |
| 29 | F | 9 8/12 | 3 weeks | 9 7/12 | | 0.93 | | 4.1 |
| 30 | F | 10 | 12 days | 10 | 21.4 | 1.03 | 0.22 | 2.9 |
| 31 | M | 11 | 5 weeks | 11 | | 1.16 | | 2.8 |
| 32 | M | 14 | 5 days | 14 | | 2.33 | | 5.1 |
| 33 | F | 14 | 8 weeks | 14 | 50.0 | 0.58 | 0.29 | 3.8 |
| 34 | M | 14 | 4 weeks | 14 | 60.0 | 1.81 | 1.09 | 3.1 |
| 35 | F | 18 | 7 days | 18 | | 1.60 | | 4.4 |
| 36 | F | 20 | 3 weeks | 20 | 49.2 | 0.67 | 0.33 | 3.3 |
| 37 | F | 20 | 2 days | 20 | | 1.12 | | 4.5 |
| 38 | F | 21 | 5 weeks | 21 | | 0.58 | | 3.8 |
| 39 | M | 22 | 3 days | 22 | 50.0 | 1.74 | 0.87 | 4.7 |
| 40 | M | 25 | 7 days | 25 | 48.6 | 0.93 | 0.45 | 4.1 |
| 41 | F | 28 | 3 days | 28 | | 1.12 | | 5.0 |

*Proportion of large islets in the pancreas.* The number of pancreatic islets which measured more than ten squares in area in any pancreas tended to be directly related to the mean area of the islets in that pancreas (figure 5). Large islets were most numerous in the acute diabetics and least numerous in the chronic diabetics (figure 6).

*Proportion of islet tissue in the pancreas.* In each group the proportion of islet tissue in the pancreas was highest in young children and tended to decrease with increasing age of the patient (figure 7). In both groups of diabetics it tended to diminish as the duration of the disease increased (figures 8 and 9).

The mean proportion of islet tissue in the diabetic subjects was significantly less than in the controls (2.45 per cent) and was significantly less in the chronic diabetics (0.67 per cent) than in the acute (1.54 per cent).

*Weight of pancreatic islet tissue.* The mean weight

TABLE 4

Controls

| Sex | Age at death (years) | Weight of pancreas gm. | Percentage of islet tissue | Weight of islet tissue gm. | Mean area of one islet (squares) |
|---|---|---|---|---|---|
| M | 3½ | 21.1 | 3.31 | 0.70 | 2.9 |
| M | 6 | 24.0 | 3.01 | 0.72 | 2.6 |
| F | 13 | 28.6 | 3.23 | 0.92 | 4.3 |
| M | 13 | 58.8 | 1.48 | 0.87 | 3.3 |
| M | 13 | 44.6 | 1.90 | 0.85 | 3.1 |
| F | 15 | 78.4 | 1.88 | 1.47 | 3.9 |
| F | 15 | 44.5 | 2.61 | 1.10 | 3.6 |
| F | 16 | 29.8 | 3.62 | 1.08 | 3.3 |
| F | 17 | 47.6 | 2.88 | 1.37 | 2.3 |
| F | 19 | | 2.89 | | 3.5 |
| F | 20 | 77.5 | 2.49 | 1.92 | 3.5 |
| F | 22 | | 2.95 | | 3.4 |
| F | 23 | 55.6 | 2.54 | 0.81 | 2.5 |
| M | 23 | 73.6 | 2.96 | 2.18 | 3.5 |
| F | 24 | | 1.88 | | 3.8 |
| M | 24 | | 2.08 | | 2.8 |
| F | 25 | | 3.11 | | 3.6 |
| M | 25 | 99.9 | 1.41 | 1.40 | 3.8 |
| F | 28 | 52.9 | 1.62 | 0.86 | 3.7 |
| M | 28 | | 1.59 | | 2.5 |
| F | 28 | | 2.41 | | 4.7 |
| F | 29 | 81.5 | 1.91 | 1.56 | 2.9 |

of the islet tissue in the diabetics was less than in the controls (1.19 gm.) and was less in the chronic diabetics (0.21 gm.) than in the eight acute diabetics (0.70 gm.) in which the pancreatic weights were known (figure 10). These differences are statistically significant, but the number of acute cases in which the pancreatic weight was known was unfortunately small. In an attempt to ascertain whether the differences were likely to be present throughout the whole group of acute diabetics, hypothetical weights of islet tissue were calculated. In figure 11 a hypothetical weight, based upon the age of the patient, was allotted to those pancreases in which the real weight was not known. In patients fourteen years of age and older, the pancreatic weight in both acute and chronic diabetics was assumed to be 40 gm.—an assumption which probably results in an underestimate of the weight of islet tissue in these acute cases. The distribution of values obtained is similar to that of the known weights of islet tissue, and suggests that in the acute diabetic group the known weights of islet tissue are representative of the group as a whole.

The weight of islet tissue tended to decrease as the duration of the disease increased (figure 12). This trend affected not only the long-established diabetics but also, with one exception, the acute diabetics (figure 13). (In figure 13, the eleven-weeks-old infant is omitted since the weight of pancreatic tissue at this age is not comparable with that of older children.)

*Proportion of β cells in pancreatic islets.* Estimation

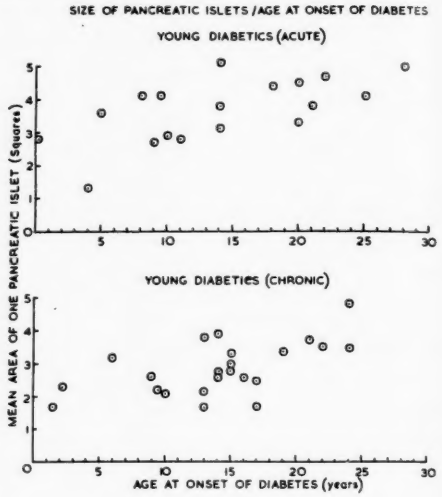
SIZE OF PANCREATIC ISLETS / AGE AT ONSET OF DIABETES
YOUNG DIABETICS (ACUTE)
YOUNG DIABETICS (CHRONIC)
MEAN AREA OF ONE PANCREATIC ISLET (Squares)
AGE AT ONSET OF DIABETES (years)

FIGURE 3

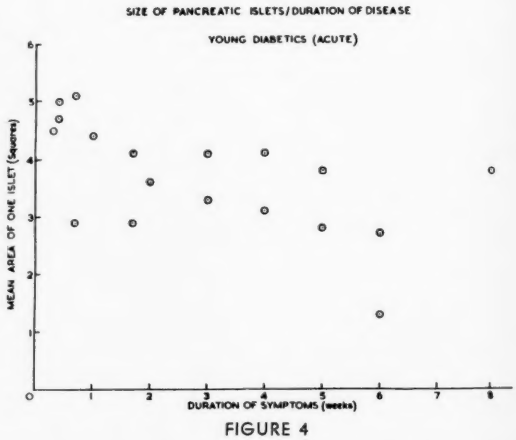
SIZE OF PANCREATIC ISLETS / DURATION OF DISEASE
YOUNG DIABETICS (ACUTE)
MEAN AREA OF ONE ISLET (Squares)
DURATION OF SYMPTOMS (weeks)

FIGURE 4

of the proportion of β cells was outside the scope of the present investigation, but observations had been made in a previous investigation on four of the present series, and differential counts were made in two additional cases. In these six cases the proportion of β cells was lower in the chronic than in the acute cases (table 5).

*Other changes in the pancreatic islets.* The islets of the diabetic subjects commonly showed degranulation of the β cells which was usually more severe in the acute than in the chronic diabetic cases. Hydropic change was less frequent in both groups. Slight lymphocytic infiltration of some of the islets was noted in three of the acute cases. The regeneration of islets described by Weichselbaum[2] was obvious in all of the acute and in ten of the chronic diabetic subjects.

TABLE 5

Proportion of α and β cells in pancreatic islets

| Sex of patient | Age of patient (years) | Duration of diabetes | Mean area of one islet (squares) | Proportions of islet cells α | β | Mean α:β cells proportions |
|---|---|---|---|---|---|---|
| F | 20 | 2 days | 4.5 | 24 | 76 | |
| M | 8 | 12 days | 4.1 | 25 | 75 | 1 : 2.7 |
| F | 14 | 8 weeks | 4.8 | 33 | 67 | |
| M | 22 | 5 years | 2.5 | 35 | 65 | |
| F | 27 | 14 years | 2.2 | 46 | 54 | 1 : 1.1 |
| F | 28 | 19 years | 2.6 | 54 | 46 | |

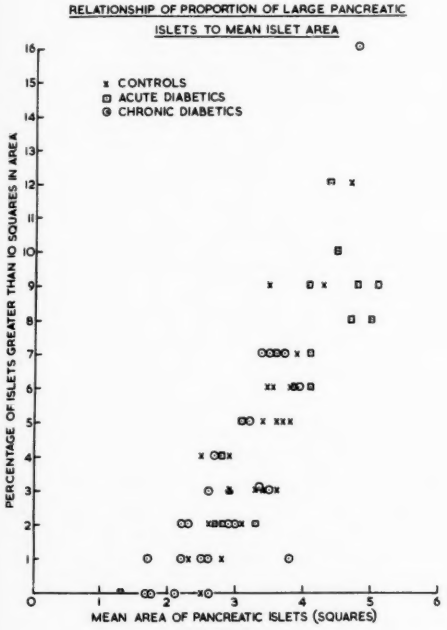
RELATIONSHIP OF PROPORTION OF LARGE PANCREATIC ISLETS TO MEAN ISLET AREA
CONTROLS
ACUTE DIABETICS
CHRONIC DIABETICS
PERCENTAGE OF ISLETS GREATER THAN 10 SQUARES IN AREA
MEAN AREA OF PANCREATIC ISLETS (SQUARES)

FIGURE 5

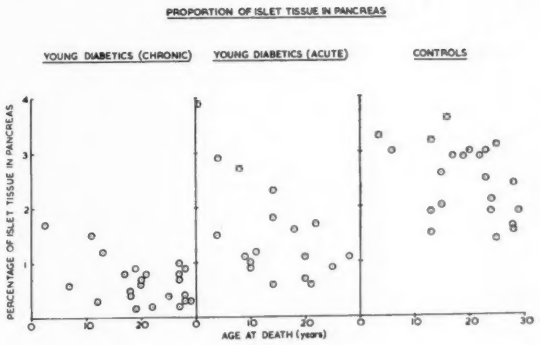


FIGURE 7

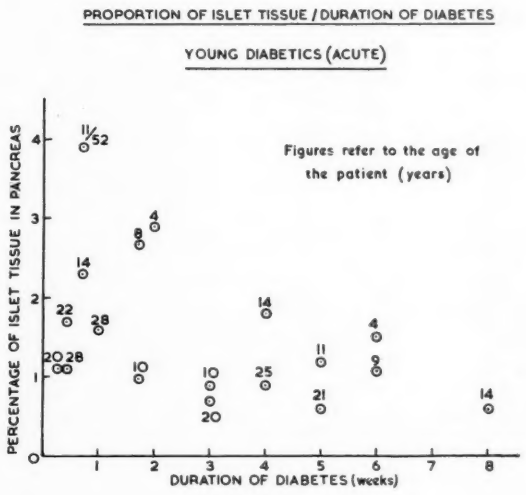
PROPORTION OF ISLET TISSUE / DURATION OF DIABETES
YOUNG DIABETICS (ACUTE)
Figures refer to the age of the patient (years)
PERCENTAGE OF ISLET TISSUE IN PANCREAS
DURATION OF DIABETES (weeks)

FIGURE 8

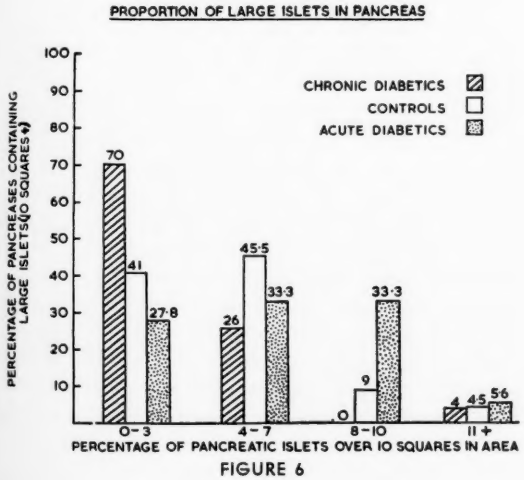


FIGURE 6

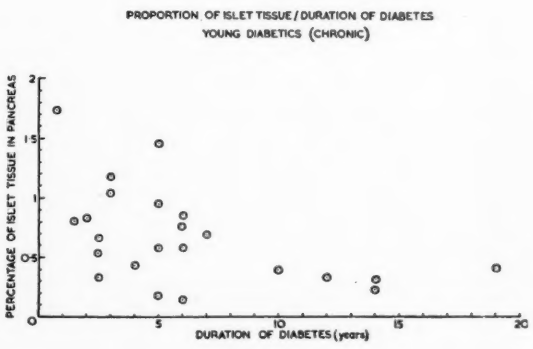
PROPORTION OF ISLET TISSUE / DURATION OF DIABETES
YOUNG DIABETICS (CHRONIC)
PERCENTAGE OF ISLET TISSUE IN PANCREAS
DURATION OF DIABETES (years)

FIGURE 9

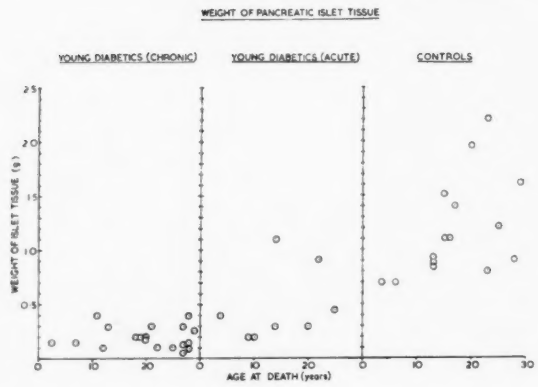


FIGURE 10

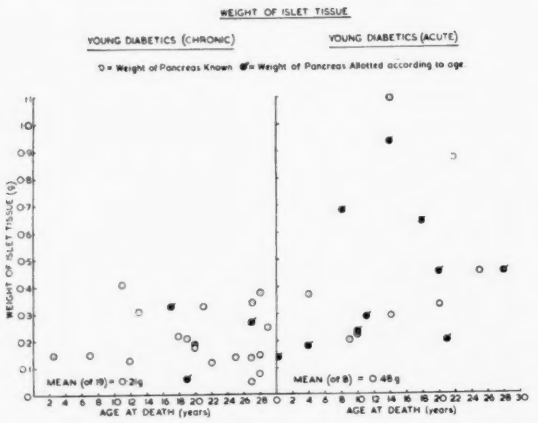
WEIGHT OF ISLET TISSUE
YOUNG DIABETICS (CHRONIC)
YOUNG DIABETICS (ACUTE)
O = Weight of Pancreas Known
Weight of Pancreas Allotted according to age
WEIGHT OF ISLET TISSUE (g)
MEAN (of 19) = 0·21g
MEAN (of 8) = 0·48g
AGE AT DEATH (years)
AGE AT DEATH (years)

FIGURE 11

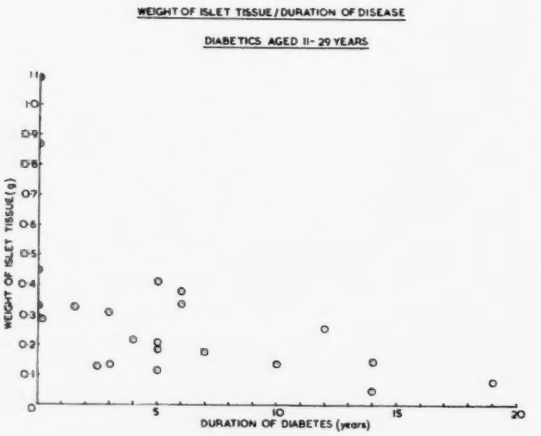
WEIGHT OF ISLET TISSUE / DURATION OF DISEASE
DIABETICS AGED 11-29 YEARS
WEIGHT OF ISLET TISSUE (g)
DURATION OF DIABETES (years)

FIGURE 12

## DISCUSSION

Experimental investigation has done much to clarify the pathogenesis of diabetes mellitus, but the causation of "essential diabetes" remains obscure.[3] In some cases, impaired function of the β cells may be associated with fibrosis or hyalinization of the islets. In others, where the islets appear normal in sections stained by hematoxylin and eosin, reduction in the amount of β cell tissue[1] and degranulation of the β cells[4] are probably responsible for the reduced insulin content of the pancreas. For these reasons, the pancreatic insulin content may be extremely low in diabetics who develop the disease during the growing period.[5] Abnormalities such as these suffice to account for the loss of control of carbohydrate metabolism, but they do not explain why the disease occurs. Any such explanation must take into account the hereditary nature of the disease.[6–8]

Diabetes can be produced experimentally either by (a) the effects of normal metabolic stress upon a subnormal amount of islet tissue, or (b) by excessive metabolic strain or by toxins acting upon a normal quantity of islet tissue. It is possible that one or both of these processes may be concerned in the hereditary influence in diabetes. If initiation of the diabetic syndrome is dependent upon hypoplasia of the islets, then the amount of islet tissue should be abnormally low at the time of onset of the disease. Opportunities to demonstrate that this is so seldom or never occur in man. If, however, the amount of islet tissue can be shown to be within normal limits shortly after the development of symptoms, it is probably safe to infer that it was not abnormally low at the time of onset of the disease. In the present series, the weight of islet tissue in three of the acute diabetics (numbers 26, 34 and 39), and the proportion of islet tissue in three additional acute cases (numbers 27, 33 and 35) in which the weight of the pancreas was not known, were within the control limits. There are other reasons to believe that, in some cases, the development of the pancreas may be normal. For example, the mean weight of the pancreases of the acute diabetics at the ages of fourteen and older was not significantly different from that of the controls, suggesting that the growth of the pancreas during childhood and early adult life was not grossly abnormal. Moreover, in the pancreas of infants born of diabetic and prediabetic mothers, the islet tissue is hyperplastic rather than hypoplastic.[9] It would therefore appear that the onset of diabetes cannot be attributed in all cases to faulty development of the pancreas.

Hyperplasia of the islet tissue was a recognized finding in some diabetic subjects even in the days when

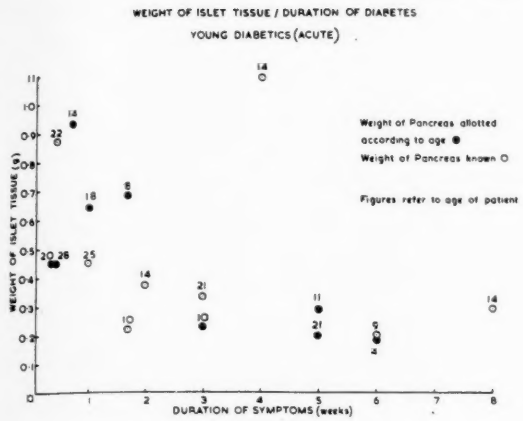
WEIGHT OF ISLET TISSUE / DURATION OF DIABETES
YOUNG DIABETICS (ACUTE)
Weight of Pancreas allotted according to age
Weight of Pancreas known
Figures refer to age of patient
WEIGHT OF ISLET TISSUE (g)
DURATION OF SYMPTOMS (weeks)

FIGURE 13

the function of the islets of Langerhans was still a matter of dispute. It would seem to be inconsistent with an illness which results from insulin deficiency, but it is nonetheless a well-substantiated phenomenon. In 1907, MacCallum[10] noted its occurrence in two children who died in diabetic coma after an illness of short duration, and suggested that it was compensatory for some extrapancreatic disturbance of carbohydrate metabolism which imposed an excessive strain upon the islets. Islet hypertrophy has since been reported in a variety of circumstances. It has been observed in the obese,[11] in rats force-fed high carbohydrate diets,[12] after perfusion of the pancreas of the dog with hyperglycemic blood,[13] in familial obese diabetic mice,[14] in rats and rabbits treated with pituitary extracts,[15,16] in rats injected with growth hormone,[17] and in rats treated with cortisone.[18] Under these circumstances, hyperplasia of the islets sometimes may be succeeded by degeneration of the β cells and glycosuria. Young[19,20] has suggested that hypersecretion of pituitary hormone, particularly growth hormone, by causing excessive stimulation and later exhaustion of the pancreatic islets, may be responsible for the diabetic syndrome in man.

Stimuli acting upon the islets may excite growth of already existing islets (hypertrophy), or formation of new islets from ducts or ductules. Weichselbaum[2] termed the latter process "regeneration," and found evidence of it in fifty-eight of 183 diabetic pancreases. Cecil[21] observed either regeneration or hypertrophy of the islets in thirty-four of 100 diabetics. In the present investigation, similar changes were seen in all of the acute and in ten of the twenty-three chronic diabetics, and were most pronounced in those cases dying shortly after the commencement of the disease. Measurement confirmed the observation that the islets were larger in the acute than in the chronic diabetics and the controls (figure 2) and that they were largest in those diabetics who died within a week of the onset of their symptoms (figure 4). The subsequent decrease in islet size suggests either that the hyperplastic stimulus may wane or that the islets may degenerate. Hydrops and degranulation of β cells, and the progressive decline in the proportion of β cells in the few cases which we examined, are both compatible with the latter possibility. Moreover, differences in percentage of islet tissue at the beginning and end of the two-month period after the commencement of the disease are proportionately greater than differences in islet size (figures 8 and 4). If our estimates are correct, therefore, some factor other than mere reduction in islet size must operate; some of the islets must have been destroyed or undergone atrophy. This does not exclude the possibility that the hyperplastic stimulus is subject to periods of remission. If, however, the remission was abrupt, temporary diabetes similar to that induced by pituitary and adrenal hormones in experimental animals might be expected to occur more often than it appears to do. The two possibilities are not mutually exclusive and both may operate; for example, hyperglycemia may produce continued islet damage after the hyperplastic stimulus which induced the diabetes has diminished in intensity.

The pattern which appears to emerge from the present observations is that diabetes in the young may be accompanied by initial hyperplasia of the islets, may occur in some patients who have a normal quantity of pancreatic islet tissue, and may result within a few weeks in diminution of islet tissue to levels which are usually met with in young chronic diabetics (figure 13); thereafter, there is a very gradual decline in the proportion and weight of islet tissue (figures 9 and 12). This succession of changes in the pancreas of young diabetics recalls the sequence of events in mature dogs treated with pituitary extracts.[22,23] The dogs are insulin-insensitive during the period of administration of the hormone, but after becoming permanently diabetic, they regain their insulin sensitivity on withdrawal of the hormone. Their islets are permanently damaged, and the pancreas has a low insulin content. The metahypophyseal diabetic dogs, therefore, resemble the insulin-sensitive diabetics of Himsworth,[24] the growth-onset diabetics of Wrenshall and his associates,[5] and the young chronic diabetics of the present series. In both groups of the young diabetics of this investigation, the pancreatic findings are not inconsistent with Young's concept

of the causation of diabetes in man. They also suggest that young diabetics dying in coma shortly after the onset of the disease resemble in some respects elderly obese diabetics. Both may exhibit insulin resistance and may possess large islets and relatively normal quantities of islet tissue.[3] These similarities are of some interest since Young attributes the clinical differences and end results of diabetes in the young and old to differences in intensity and duration of pituitary stimulation.

The part played by stress in the causation of diabetes has been reviewed so recently[25] that it is unnecessary for us to make any general comment on it, but we can hardly conclude this consideration of diabetes in the young without mention of infectious illness as a possible islet injuring or stimulating factor, since it commonly precedes clinical manifestations of the disease and may quadruple the insulin requirements in established diabetes.[7] It might exert its effect either by a direct toxic action upon the pancreatic islets, or indirectly by increasing the metabolic needs of the patient. In such a manner, toxic infection might prove to be the last straw to islets under stress from a constitutional metabolic disorder. Our results merely suggest that a metabolic disorder which causes initial hyperplasia of the pancreatic islets and later degeneration of the β cells may exist, and that it may be a factor in the causation of diabetes even in patients with normal amounts of islet tissue.

## SUMMARY

Quantitative observations on the pancreatic islet tissue were made on forty-one young diabetics and twenty-two control subjects. Eighteen of the diabetic patients died within eight weeks of the onset of symptoms ("acute"), and twenty-three died at intervals ranging from nine months to nineteen years after the commencement of the disease ("chronic").

The size of the islets, and the proportion and weight of islet tissue were greater in the acute than in the chronic diabetic cases. In both groups these values tended to decline with increasing duration of the disease, rapidly in acute and slowly in chronic subjects.

In acute diabetics the islets were larger than in the control patients, and in some of them the proportion and weight of islet tissue were normal.

The bearing of these observations on the diabetic syndrome in man is discussed.

## SUMMARIO IN INTERLINGUA

*Observationes Relative Al Histo Del Insulas Pancreatic In Juvene Diabeticos*

Observationes quantitative relative al histos del insulas pancreatic esseva effectuate in quaranta-un juvene diabeticos e vinti-duo subjectos de controlo. Dece-octo del diabeticos moriva intra octo septimanas post le declaration del symptomas (lor casos esseva "acute"), e vinti-tres moriva post intervallos de inter nove menses e dece-nove annos a partir del comenciamento del morbo (lor casos esseva "chronic").

Le dimensiones del insulas e le proportion e le peso del histo insular esseva plus grande in le acute que in le chronic casos de diabete. In ambe gruppos, ille valores tendeva a declinar in le curso del morbo—rapidemente in le casos acute e lentemente in le casos chronic.

In le subjectos con diabete acute le insulas esseva plus grande que in le subjectos de controlo. In certe subjectos con diabete acute le proportion e le peso de histo insular esseva normal.

Es discutite le signification de iste factos pro le syndrome diabetic in humanos.

## ACKNOWLEDGMENT

It is a pleasure to acknowledge our indebtedness to those who have aided us in this investigation: to our colleagues in Edinburgh, to Professors D. F. Cappell, J. B. Duguid, and T. Symington, and to Dr. A. M. Macdonald who generously placed pancreatic material at our disposal; to Dr. B. Woolf for statistical advice; to Mr. Ian Bell who was responsible for much of the histological preparations; and to members of the Medical Photography Unit, University of Edinburgh, for the illustrations.

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A. S. Earle, G. F. Cahill and Hoar (*Ann. Surg. 146*: 124, 1957), by employing the determination of blood pepsinogen levels, endeavored to determine whether or not glucagon played a role in gastric secretory activity. Mongrel dogs weighing 8 to 15 kg. were fed a standard ration until eighteen hours before the beginning of each experiment. Amorphous glucagon was administered intravenously during pentobarbital anesthesia and femoral vein blood was analyzed at intervals for glucose and pepsinogen levels. The rapid injection of 200 μg. of glucagon resulted in glucose values between 184 and 232 mg. per cent, reached between fifteen and thirty minutes after injection. The simultaneous decrease in blood pepsinogen level from 300 to 260 units, though probably significant, indicated the necessity for testing the effects of glucagon administered by continuous drip.

Infusions lasting from two to four hours, with dose rates ranging between 2.5 and 3.3 μg. per minute, resulted in significant decreases in blood pepsinogen levels during the induced hyperglycemia. The average control level for blood pepsinogen of 420 units fell to 320 and 250 units after one half and two hours of glucagon infusion, respectively. During the hour after the completion of infusion the blood sugar values returned to normal, but the blood pepsinogen levels remained low. The fact that no significant alteration of normal blood glucose or pepsinogen level occurred during anesthesia alone eliminated the possibility that pentobarbital was responsible for depressing gastric activity. In order to learn whether the vagi influenced gastric activity after glucagon, the infusion was repeated in animals that had undergone vagotomies one week before. Although the blood pepsinogen level dropped following vagisection, a further significant drop was observed following the infusion of glucagon over a two-hour period. Intravenous infusion of glucose was used in an additional experiment to determine whether the hyperglycemia was responsible for the drop in pepsinogen level. The animals showed significant decreases in blood pepsinogen levels comparable to those seen following glucagon injection.

The injection of glucagon into human subjects showed results essentially similar to those seen in the experimental animals. The rapid injection of 0.8 mg. elicited a moderate rise in blood glucose (74 mg. per cent to 134 mg. per cent) and a decrease in blood pepsinogen from 360 units per milliliter to 335 units per milliliter. Subjects 2 and 3 received infusions over a two-hour period and the gastric acid collections were evaluated for hourly volume and acidity. Subject 2, a normal volunteer, was given 1.75 mg. of glucagon during two hours and subject 3 with duodenal ulcer, associated hyperacidity, and elevated blood pepsinogen was given 2.5 mg. of glucagon during two hours. The blood pepsinogen levels of both subjects dropped more than 50 units and the milliequivalents of total acid decreased.

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# Insulin Antagonists in Blood of Juvenile Diabetics with Arterial Disease

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A study of forty-seven juvenile onset diabetics with a history of more than fifteen years (Downie and Martin, 1958) has shown that the presence of arterial complications could not be related either to control of the diabetes or to its severity. Accordingly it was decided to study these patients with regard to the presence or absence in the blood of the insulin inhibitory factor described by Baird and Bornstein (1957).

Assays carried out on the blood of untreated diabetic patients with an onset at less than eighteen years of age have shown that neither insulin-like or insulin-inhibitory activity is present in their plasma (Baird and Bornstein, 1957; Bornstein and Hyde, unpublished data). It is reasonable to assume, then, that this group of patients would be particularly suitable for the present study.

## MATERIALS AND METHODS

The patients studied comprised an unselected group of thirteen patients showing either severe retinal changes or nephropathy or both, and twelve patients showing either minimal or no evidence of any vascular degeneration. Blood was taken from these patients early in the morning prior to the first dose of insulin, the plasma recovered after centrifugation and then either immediately extracted or else deep-frozen and then extracted at convenience.

The extraction technic using ethanol-butanol-toluene at *p*H 2 described by Baird and Bornstein (1957) was used throughout, and the inhibitory material recovered from the first residue ($R_1$).

The activity of the first residue ($R_1$) was tested on rat hemidiaphragms using the technic of Brown et al. (1952) at a glucose level of 200 mg./100 ml.; where extract was added it was at a concentration of the equivalent of 1 ml. original plasma/milliliter buffer.

The rats used were of Baker Institute stock and it has been found that the most consistent results are obtained using males between 110 and 130 gm. weight.

## RESULTS

The over-all results obtained are shown in table 1. It is seen that in all cases showing severe retinopathy or nephropathy inhibitory activity was demonstrated in the plasma residues after extraction of the insulin, whereas in only four out of twelve patients showing little or no vascular damage could any such activity be demonstrated.

Owing to shortage of animals, individual sera have not been assayed to statistical significance but when the two groups are considered it is seen that the inhibitory activity of the sera from the group with arterial involvement is significantly increased over that of the control series.

## DISCUSSION

The demonstration that the first residue $R_1$ is more active in complicated than in otherwise comparable noncomplicated juvenile onset diabetic patients makes it very tempting to assume that this inhibitory activity is responsible for the vascular degeneration.

If the finding of no inhibitory activity in the plasma of seventeen juvenile diabetics investigated at the onset of the disease holds for all such patients, then secretion of the active material in fraction $R_1$ must become established at a later date and raises the interesting speculation that division between juvenile diabetics who become complicated and those who do not is dependent on hypersecretion of an appropriate insulin antagonist.

However, it is possible that the apparent increase in the circulating "inhibitor" is entirely due to the presence of vascular disease, particularly if nephropathy is present or may simply be the reflection of the somewhat higher insulin dosage of the complicated group as large amounts of this substance have been shown to be present in the plasma of some insulin resistant diabetics, particularly when in coma.

It can also be seen that some patients showing minimal or no complications have had demonstrable activity in fraction $R_1$. However, the technic used is not an assay in the true quantitative sense and it is possible that the activity circulating in these patients may be appreciably less than in the complicated patients.

Although at this stage the significance of these findings cannot be assessed, they indicate a possible lead to

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TABLE 1

| Patient | Sex | Age of onset (left) and duration of diabetes in years | | Retinopathy | Albuminuria | Mean effect of fraction R, on rate of glucose utilization by rat diaphragm mg./gm./hr.* | |
|---|---|---|---|---|---|---|---|
| A. Control series | | Twelve patients | | | | | |
| J.W.† | F | 12 | 20 | + | 0 | — 0.3 | (4) |
| J.S. | F | 6 | 32 | 0 | 0 | 0.0 | (4) |
| L.B. | F | 8 | 33 | 0 | 0 | 0.0 | (3) |
| M.V. | F | 5 | 31 | 0 | 0 | 0.0 | (2) |
| P.S. | F | 17 | 39 | 0 | 0 | + 0.3 | (3) |
| M.B. | M | 5 | 26 | 0 | 0 | + 0.1 | (3) |
| M.F. | F | 9 | 27 | 0 | 0 | + 0.2 | (3) |
| N.M. | F | 16 | 20 | 0 | 0 | + 0.1 | (3) |
| R.A. | M | 15 | 20 | 0 | 0 | 0.0 | (3) |
| M.H. | F | 6 | 29 | 0 | 0 | — 0.65 | (2) |
| J.W. | F | 4 | 28 | 0 | 0 | — 0.2 | (3) |
| K.S. | M | 12 | 23 | 0 | 0 | — 0.3 | (3) |
| Mean age of onset and duration | | 9.6 | 27.3 | | Mean inhibition + S.E.M. | — 0.05 ± 0.058 | (36) |
| B. Complicated series | | Thirteen patients | | | | | |
| K.B.‡ | M | 9 | 17 | +++ | +++ | — 0.6 | (4) |
| N.D.‡ | F | 15 | 21 | ++ | ++ | — 0.85 | (4) |
| P.O.'S. | M | 15 | 22 | + | + | — 3.5 | (3) |
| N.B. | M | 16 | 22 | + | +++ | — 1.25 | (4) |
| G.B. | F | 12 | 27 | + | 0 | — 0.60 | (3) |
| R.S. | F | 11 | 20 | ++ | 0 | — 0.30 | (3) |
| N.S. | M | 7 | 21 | + | + | — 0.60 | (4) |
| V.D. | M | 2 | 28 | ++ | +++ | — 0.90 | (2) |
| R.F. | F | 4 | 28 | + | ++ | — 0.40 | (4) |
| R.B. | M | 18 | 27 | + | + | — 0.3 | (4) |
| D.G. | F | 12 | 22 | ++ | +++ | — 0.4 | (3) |
| J.K. | M | 18 | 26 | + | ++ | — 0.3 | (4) |
| V.B. | F | 10 | 20 | + | 0 | — 0.3 | (4) |
| Mean age of onset and duration | | 11.5 | 23.2 | | Mean inhibition + S.E.M. | — 0.75 ± 0.125 | (46) |

*Number of diaphragms used are in parentheses.
†Included in control series in spite of some retinopathy owing to excellence of rest of vascular system.
‡Not included in series studied by Downie and Martin.
For the changes in glucose uptake by diaphragms exposed to fraction R, from uncomplicated and complicated diabetics: $n = 80$; T is 4.622; $P<0.001$. Analysis of variance of ages of onset shows that both groups belong to the same series.

the causation of arterial degeneration in diabetes. This possibility is being further investigated.

## SUMMARY

1. Two groups of juvenile onset diabetics of long standing have been investigated as to the presence or absence of plasma insulin antagonists. Thirteen had arterial complications (retinopathy and/or nephropathy). Twelve were uncomplicated.

2. It was shown that there is a greater concentration of antagonist present in the complicated group.

3. The significance of this finding is discussed.

## SUMMARIO IN INTERLINGUA

*Antagonistas A Insulina In Le Sanguine De Patientes Con Diabete Juvenil E Morbo Arterial*

1. Duo gruppos de patientes con diabete de longe durantia e declaration juvenil esseva investigate con respecto al presentia o absentia de antagonista a insulina in le plasma. Le dece-tres patientes del prime gruppo habeva complicationes arterial, i.e. retinopathia e/o nephropathia. Le dece-duo casos del secunde gruppo esseva noncomplicate.

2. Esseva monstrate que le antagonista esseva presente in plus alte concentrationes in le gruppo con complicationes.

3. Le signification de iste constatation es discutite.

## ACKNOWLEDGMENT

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# Impaired Glucose Tolerance and Glucosuria Induced in Man by Repeated Injections of Glucagon

*Theodore B. Van Itallie, M.D., Jean-Pierre Felber, M.D., Joseph Hoet, M.D., and Albert E. Renold, M.D., Boston*

When glucagon is given in a single injection or by infusion it induces a transient hyperglycemia by stimulating hepatic glycogenolysis.[1-3] Under such circumstances, inhibition of peripheral glucose uptake does not occur.[4-7] Thus, despite earlier opinion to the contrary,[8-9] glucagon does not appear to act directly as an insulin antagonist.

On the other hand, it has been reported that when repeated injections of glucagon are given to force-fed rats,[10] cats and dogs,[11] and patients with rheumatoid arthritis,[12,13] glucosuria, hyperglycemia, ketosis and a "protein-catabolic" state may ensue.

Ezrin and associates[12] have attributed the hyperglycemia induced by repeated injections or infusions of glucagon to an increased production of new glucose from amino acids as well as increased hepatic glycogenolysis. They believe increased gluconeogenesis from protein to play the most important role in the genesis of the diabetic-like state induced by glucagon. Elrick, Rachiele, and Head[11] have suggested that glucagon-induced diabetes should be placed in the category of conditions such as occur in force feeding, in which transient hyperglycemia and glucosuria are produced without impairment of glucose utilization.

In an attempt to obtain more information about the metabolic consequences of prolonged administration of glucagon and, in particular, about its effect on carbohydrate homeostasis, glucagon was administered by repeated injection to a series of normal human subjects maintained on constant diets while on a metabolic ward. The results show that, in man, repeated injections of glucagon can induce a transient diabetic-like state in which the glucosuria results from impaired tolerance to ingested carbohydrate. In several respects, glucagon-diabetes resembles the diabetic-like state classically induced by carbohydrate deprivation.

**Presented by title at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco June 21-22, 1958.**

From the Department of Nutrition, Harvard School of Public Health, and the Departments of Medicine, Harvard Medical School and the Peter Bent Brigham Hospital, Boston, Massachusetts. Dr. Van Itallie's present address: St. Luke's Hospital, New York, New York.

## PROCEDURE

The glucagon* used was a crystalline, insulin-free preparation made by the method of Staub et al.[14] It was administered at six-hourly intervals in amounts of 1.3 to 4.0 mg. for periods of two to five days.† Usually the glucagon was administered intramuscularly, but in one experiment (J.O'B.), the subject was given three morning doses and two afternoon doses intravenously.

Three young men, aged 20, 22 and 27, and one young woman, aged twenty-five, served as volunteer subjects. All the subjects were in good health. They were studied on the Metabolic Ward of the Peter Bent Brigham Hospital, with weighed constant diets and quantitative collections of urine. Feces were not collected.

The experimental periods involving glucagon administration were preceded by a glucose tolerance test and by a control period of four to five days during which a constant diet containing at least 250 gm. of carbohydrate per day and calories adequate to maintain body weight was consumed. Control blood and urinary collections were made during this same period.

During the experimental period which lasted two to five days, the subject remained on his constant diet; however, in addition, glucagon was administered every six hours in amounts of 1.3 to 4.0 mg. After completion of the experimental period, the glucose tolerance test was repeated.

One subject (J.O'B.) had a three-day postexperimental control period during which intake remained the

* Supplied through the courtesy of Dr. W. R. Kirtley of Eli Lilly and Company, Indianapolis, Indiana.

† Three of the subjects presented in varying degrees one or more of the following symptoms during the period of glucagon administration: anorexia, nausea, bloating and, in one instance, vomiting.

same as in the first two periods.

In two subjects (one of the subjects previously described and an additional young man, aged twenty-eight, not maintained on a strict metabolic balance regimen), measurements of maximal tubular reabsorptive capacity for glucose (glucose $T_m$) were obtained before and after forty-eight and sixty hours of glucagon administration respectively at a rate of 4 mg. every six hours.

Three subjects had fractional urine collections during at least one twenty-four-hour interval of the experimental period.

Glucose tolerance tests were made in the morning approximately fourteen hours after the previous meal. In two experiments, 0.5 gm. of glucose per kilogram body weight was given intravenously over a period of thirty minutes and capillary (finger tip) and antecubital venous blood sugar levels were measured at fifteen-minute intervals for two hours. Control blood sugar levels also were determined.

In one experiment, glucose was administered intravenously by constant (Bowman pump) infusion at a rate of 0.5 gm. per kilogram body weight per hour for a total of eight hours. Following a control determination, venous blood sugar levels and urinary glucose excretion were measured at hourly intervals during the infusion.

In one experiment, 1.3 mg. of glucagon was administered intravenously (1.0 mg. of glucagon per ml. of diluent) before breakfast and in the early afternoon before luncheon on the first and third day of the experimental period, and before breakfast only during the fifth day of the experimental period. Capillary glucose and antecubital venous glucose and ketone levels were measured at thirty and forty-five minutes after each injection and control blood samples also were obtained.

Dietary constituents were determined by means of standard tables and analyses of representative portions. Calorie intake was calculated from weighed portions and refusals on the basis of appropriate tables.

Urinary glucose was measured enzymatically, as described by Froesch and Renold.[15] Glucose was measured in whole blood by Somogyi's method[16] as modified by Nelson.[17] All determinations were done in duplicate on 0.2 ml. samples. Blood ketones were determined by the method of Michaels et al.[18]

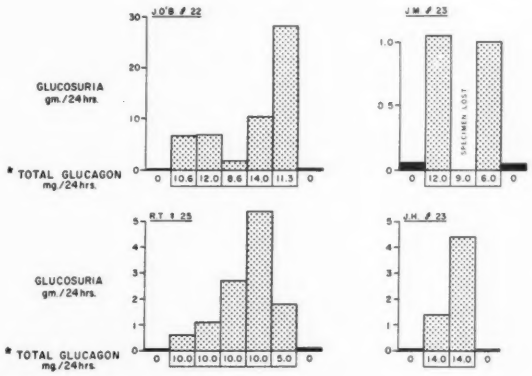
Glucose $T_m$ was determined according to the method of Smith et al.[19] as modified by Froesch and associates,[20] while glomerular filtration rate was measured by means of insulin clearance.[21]

Capillary blood was obtained by direct pipetting from a finger tip after cutaneous puncture.

## RESULTS

All four subjects displayed a significant degree of glucosuria while glucagon was being given (figure 1). There was considerable individual variation in the glucosuric response, the maximal glucose output for each subject per twenty-four hours falling within the range of 1.1 to 28.2 gm. (normal $<0.25$ gm.).

The hyperglycemic response to glucagon given intravenously was measured in one subject as the experimental period progressed (figure 2). At the same time, blood ketones were determined. It will be noted that the blood sugar never rose to levels higher than 130 mg./100 ml. after glucagon, whether this substance was administered intravenously or intramuscularly.* The


J.O'B. ♂ 22
J.M. ♂ 23
GLUCOSURIA gm./24 hrs.
* TOTAL GLUCAGON mg./24 hrs.
0 10.6 12.0 8.6 14.0 11.3 0
SPECIMEN LOST
0 12.0 9.0 6.0 0
R.T. ♀ 25
J.H. ♂ 23
GLUCOSURIA gm./24 hrs.
* TOTAL GLUCAGON mg./24 hrs.
0 10.0 10.0 10.0 10.0 5.0 0
0 14.0 14.0 0

*Given in four equal six-hourly injections

**FIG. 1. Glucosuria in four subjects during treatment with repeated injections of glucagon. Each vertical bar represents a twenty-four-hour collection period.**

pattern of response to glucagon changed as the experimental period progressed and also varied with the time of day. On the third and fifth days of the experimental period (figure 2B and 2C), the fasting blood glucose level was substantially lower than on the first day (figure 2A). At the same time, the total glucose increment was greater on the mornings of the third and fifth days, the capillary-venous (C-V) glucose differences became smaller, and the slopes of the disappearance curves after the hyperglycemic peaks were reached were more gradual. Blood ketone levels on the mornings of the third and fifth days were appreciably higher than on the first day. In contrast, the early afternoon responses to glucagon (pre-luncheon) on the

* Blood sugar levels were determined at hourly intervals for three hours following intramuscular injection of 4.0 mg. of glucagon in subject J. O'B. and did not exceed 118 mg./100 ml.

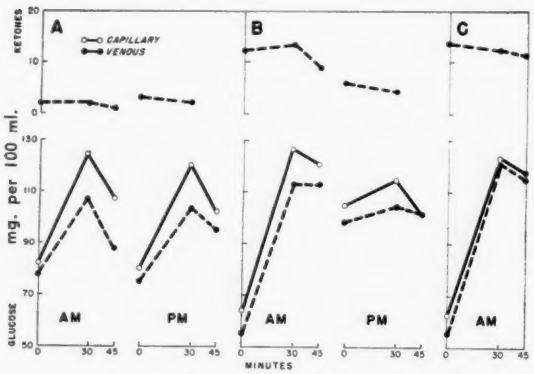



FIG. 2. (Subject J. O'B.) Changes in concentration of blood ketones and glucose after intravenous glucagon (1.3 mg.) on different days. A. First day of glucagon treatment. B. Third day of glucagon treatment. C. Morning of fifth day of glucagon treatment. Morning and afternoon injections at zero time were given before breakfast and luncheon respectively.

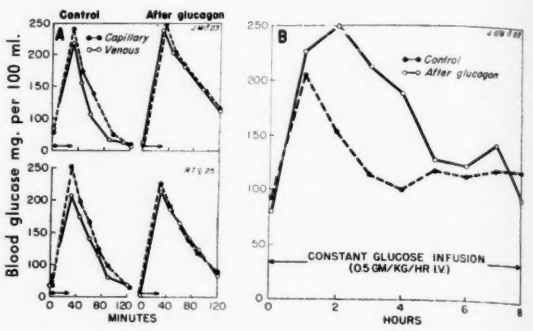



FIG. 3. A. Intravenous glucose tolerance tests in two subjects before and after glucagon treatment. Capillary and venous glucose concentrations were measured. One-half gram glucose per kilogram body weight infused over a thirty-minute period.
B. (Subject J. O'B.) Changes in blood glucose concentration during prolonged constant glucose infusion, before and after glucagon treatment.

first and third days showed small rises in blood glucose concentration, appreciable C-V differences, and a rapid decrement, while blood ketone levels in the afternoon of the third day were considerably lower than on the morning of that day.

Values for glucose $T_m$ were measured in two normal subjects before and after forty-eight and sixty hours of repeated glucagon injections. In the first instance the $T_m$ (mean of four periods) decreased by 6 per cent after glucagon, and in the second instance the $T_m$ (mean of four periods) increased by 11 per cent after glucagon. These changes were not considered to be significant.

The effect of repeated injections of glucagon on tolerance to intravenously administered glucose is shown in figure 3. During the short intravenous glucose tolerance tests (Part A), C-V glucose differences were measured in order to determine the effect of glucagon treatment on peripheral glucose uptake. In each instance, C-V glucose differences diminished in the postglucagon tolerance test, while at the same time the slopes of the disappearance curves became more gradual.

During the eight-hour glucose infusion after glucagon treatment (Part B of figure 3), 56.1 gm. of glucose were lost in the urine while only 7.6 gm. of glucose appeared in the urine during the preglucagon eight-hour infusion.

During glucagon treatment, fasting blood glucose levels tended to remain normal or fall below their control levels, as shown in table 1.

The time at which maximal glucosuria occurred was determined by obtaining four six-hour urine collections over a twenty-four-hour period during glucagon treatment. The results are shown in figure 4. In every instance, the glucosuria occurred principally in the morning after breakfast. Losses of glucose in the urine were negligible at other times.

TABLE 1

Fasting venous blood glucose levels (milligrams/100 ml.) before and during glucagon treatment (previous dose of glucagon six or more hours before blood sampling)

| | R.T. | J.O'B. | J.M. | J.M. |
|---|---|---|---|---|
| Before glucagon treatment | 82 | 92 | | 56 |
| | 79 | 93 | 70 | 58 |
| | 86 | 79 | 73 | 60 |
| During glucagon treatment | 52 | 78 | 56 | 46 |
| | 45 | 52 | 70 | 42 |
| | | 55 | | |
| | | 60 | | |
| | | 55 | | |

At the same time as the maximal glucosuria occurred, there was a marked impairment of glucose tolerance. This is shown in figure 5, where the responses in venous glucose level to a measured, identical breakfast containing 63 gm. of carbohydrates before and after glucagon are compared. Before glucagon administration, there was virtually no rise in venous glucose after the breakfast; after three days of glucagon treatment venous glucose rose to diabetic levels following ingestion of breakfast, and appreciable glucosuria occurred simultaneously.

## DISCUSSION

In attempting to account for the glucosuria during glucagon treatment, several possible explanations were

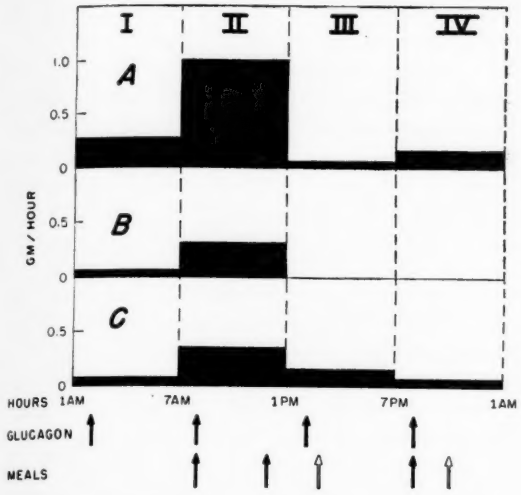



FIG. 4. Quantity of glucose in fractional urine collections from three normal subjects (A, B, and C) given equal injections of glucagon every six hours. Solid black arrows represent regular meals and light arrows snacks.

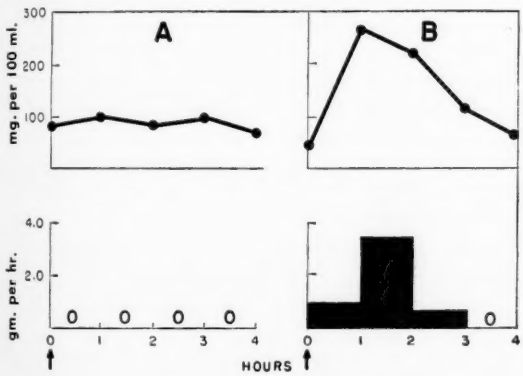



FIG. 5. Changes in blood glucose (mg./100 ml.) and glucosuria (gm./hr.) in a normal subject after breakfast. A. Before glucagon treatment. B. During glucagon treatment. Arrows represent ingestion of an identical breakfast containing 63.0 gm. of carbohydrate.

considered. It was shown that the glucosuria could not have been caused by an excessive hyperglycemia directly induced by glucagon since the blood sugar after glucagon administration never exceeded 130 mg./100 ml., considerably below the renal threshold for glucose. Root[22] has described an increasingly hyperglycemic response to glucagon associated with increased concentration of glycogen in liver in rabbits given repeated injections of glucagon. In the human subject studied here from the standpoint of responsiveness of blood glucose to glucagon, there was only a slight increase in the height of the glucagon-induced hyperglycemia after five days of glucagon treatment.

The possibility of an altered renal threshold to glucose also was considered. Elrick and associates[23] have reported on changes in renal clearance of sodium, chloride, potassium, and inorganic phosphate, although they have not described an effect of glucagon on excretion of glucose by the kidney. The two subjects in whom glucose $T_m$ was measured before and during glucagon treatment did not show any significant change in maximal tubular reabsorptive capacity for glucose; hence, it was believed that a renal effect of glucagon to account for the glucosuria was unlikely.

Cahill et al.[24] have demonstrated increased hepatic glucose-6-phosphatase activity secondary to the increased phosphorylase activity following glucagon administration in the dog. The possibility was entertained that impaired tolerance to dietary carbohydrate might have occurred because of temporary inability of the liver to retain glucose removed from blood. Although there is no evidence to rule out such an effect as a contributing factor, it seems more reasonable to account for the impaired tolerance to carbohydrate induced by glucagon treatment by a diminished rate of glucose removal by the extrahepatic tissues. The decreased peripheral C-V glucose differences measured during postglucagon glucose tolerance tests suggest that the principal site of impaired tolerance may have been in the periphery.

Thus, it would appear that glucagon affects carbohydrate metabolism by at least two mechanisms. The first mechanism is well understood and entails a rapid glycogenolytic response in the liver with consequent transient hyperglycemia. During this time, impairment of peripheral glucose utilization does not occur. The second mechanism is a delayed one in which impaired tolerance to glucose is demonstrable within less than eight hours after a single injection of glucagon.[25] The degree of impairment of glucose tolerance is particularly marked after an overnight fast during which an injection of glucagon has been administered. Conversely, ingestion of food alleviates the impaired glucose tolerance, at least in part.

The diabetic-like state induced by glucagon differs from spontaneous diabetes mellitus, steroid diabetes and metahypophyseal diabetes in that the fasting blood sugar is normal or low. It most closely resembles so-called hunger or starvation diabetes in that the fasting blood sugar remains normal or low, and liver glycogen is depleted.

The mechanism causing hunger diabetes still is not well understood.[26] Impaired glucose tolerance in hunger diabetes presumably represents an attempt by the body to conserve carbohydrate so that its own stores of protein will not have to be raided to provide indispensable glucose needed by the organism. When glucagon is given, the only mobilizable stores of carbohydrate also are depleted (although there need be no net loss of carbohydrate from the body). It seems likely that glucagon-induced "diabetes" may result from the body's attempt to maintain the blood sugar at a normal level in spite of drastic depletion of liver glycogen.

Another possibility is that glucagon administration directly inhibits insulin formation or release.[27] In any event, the impaired tolerance to glucose induced by glucagon would seem to be due to (1) a decrease in circulating insulin, (2) an increase in the secretion of insulin antagonists, or (3) a decreased tissue sensitivity to insulin.

No evidence is obtained in the present studies that appreciable hyperglycemia is present except after ingestion of carbohydrate. The fractional urine collections correlated with blood sugar determinations, as illustrated in figure 5, suggest that the glucosuria induced by repeated injections of glucagon in this series was largely, if not entirely, alimentary in origin.

## SUMMARY AND CONCLUSIONS

Crystalline glucagon was administered by repeated injection to four normal human subjects maintained on constant diets. The glucagon was given in amounts of 1.3 to 4.0 mg. intramuscularly every six hours for two to five days.

All four subjects developed significant glucosuria, ranging up to 28 gm. per twenty-four hours, during glucagon treatment. Fractional urine collections showed the glucosuria to occur principally after the morning meal. During the period of glucagon administration, fasting blood sugar values were normal or below their usual levels, but tolerance to ingested and intravenously administered glucose became impaired. Decreased capillary-venous glucose differences during intravenous glucose tolerance tests and during glucagon-induced hyperglycemia suggested that the rate of glucose assimilation by peripheral tissues had been reduced by glucagon treatment.

In one subject who displayed marked glucosuria, glucagon treatment also was associated with morning hyperketonemia.

Glucose $T_m$ determinations in two subjects before and after glucagon administration disclosed no significant change in maximal tubular reabsorptive capacity for glucose.

It is concluded that, in man, repeated injections of glucagon can induce a transient diabetic-like state in which the glucosuria results from impaired tolerance to ingested carbohydrate. A possible relationship of this phenomenon to the "diabetes" caused by carbohydrate deprivation is discussed.

## SUMMARIO IN INTERLINGUA

*Derogation Del Tolerantia De Glucosa E Induction De Glucosuria In Humanos Per Repetite Injectiones De Glucagon*

Glucagon in forma crystallin esseva administrate per repetite injectiones a quatro normal subjectos human qui esseva mantenite con dietas constante. Le glucagon esseva administrate in quantitates de inter 1,3 e 4,0 mg per via intramuscular a intervallos de sex horas durante periodos de inter duo e cinque dies.

Omne le quatro subjectos disveloppava grados significative de glucosuria, in concentrationes de usque a 28 g per vinti-quatro horas, in le curso del tractamento con glucagon. Fractional collectiones de urina monstrava que le glucosuria occurreva principalmente post le repasto de matino. Durante le periodo del administration de glucagon, le valores pro sucro sanguinee in stato jejun esseva normal o infra lor nivellos usual, sed le tolerantia pro glucosa administrate per via oral o intravenose deveniva derogate. Reducite differentias de glucosa inter capillares e venas durante tests de tolerantia pro glucosa intravenose e durante hyperglycemia inducite per glucagon suggereva que le assimilation de glucosa per le histos peripheric habeva essite relentate durante le tractamento con glucagon.

In un subjecto, qui monstrava grados marcate de glucosuria, le tractamento con glucagon esseva etiam associate con hypercetonemia matinal.

Determinationes de $T_m$ pro glucosa in duo subjectos ante e post le administration de glucagon revelava nulle significative alteration del maximal tubular capacitate reabsorptive pro glucosa.

Es concludite que repetite injectiones de glucagon in humanos pote inducer un transiente stato diabetoide in que le glucosuria resulta ab le derogate tolerantia pro ingerite hydratos de carbon. Es discutite un relation possibile inter iste phenomeno e le "diabete" causate per privation de hydrato de carbon.

## ACKNOWLEDGMENT

This investigation was supported in part by Grant No. A-1197 (later A-2416) from the National Institute

of Arthritis and Metabolic Diseases, U.S. Public Health Service; the John A. Hartford Foundation, Inc., New York City; Eli Lilly and Company, Indianapolis, Indiana; and the Fund for Research and Teaching, Department of Nutrition.

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Although the phosphogluconic acid cycle (in which a number of pentose phosphates occur as intermediates) was first demonstrated in plants and bacteria, there has now accumulated abundant evidence to indicate that similar biochemical processes are present in mammalian tissues. The occurrence of pentosurias in humans associated with congenital defects, drug ingestion, and neurological disease suggests that pentoses are of fundamental biological importance. The papers of Segal, Wyngaarden, and Foley represent one of the first attempts to clarify pentose metabolism in man, and, additionally, to offer supportive evidence obtained in the human of the membrane theory of insulin action.

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# Some Statistical Investigations in Diabetes Mellitus

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*with statistical collaboration of L. J. Smid, Ph.D., Groningen, Netherlands*

The purpose of the statistical investigations discussed in this paper was to elucidate among known diabetic subjects: (1) the age group at which the female predominance is especially manifest; (2) the age group at which "diabète maigre" and "diabète gras" occur; (3) the frequency of "diabète maigre" and "diabète gras" in males and females; (4) the significance of the number of pregnancies and of obesity as promoting factors in the pathogenesis of diabetes mellitus; (5) the cause of the female predominance.

The investigations are based on the data available from patients visiting the outpatient department for diabetes of the Clinic for Internal Diseases, University Hospital of Groningen. This is a free clinic. On Jan. 1, 1956, a total of 773 male and 1,379 female patients were under treatment, all from the three northern provinces of the Netherlands. They may be considered as representative of this part of the country.

The visitors to the outpatient department are older than twelve years. There is no further selection regarding the severity of the diabetes, sex, or age at which they come under treatment and time of onset of the diabetes. Most, however, are treated from the beginning of the disease, and then remain under regular inspection. The aim of the treatment with diet or with diet and insulin, is to reduce the blood sugar content to values below 200 mg. per 100 ml., to decrease the glucosuria to minimal amounts, to avoid acetonuria and to prevent hypoglycemia. At first, obese diabetics are treated by reducing the caloric intake to about 1,200 calories, since it is well known that an adequate weight reduction considerably improves carbohydrate tolerance.

Figure 1 represents the age distribution of the patients. This more or less corresponds with that found in other clinics. In contrast to the findings of Spiegelman and Marks,[1] who found a marked predominance of females as early as after the twenty-fifth year (20 per cent between twenty-five and thirty-four years and 100 per cent between forty-five and sixty-four years), in our clinic the number of male patients younger than forty is somewhat higher than that for the females. Over forty there is the traditional marked predominance of women.

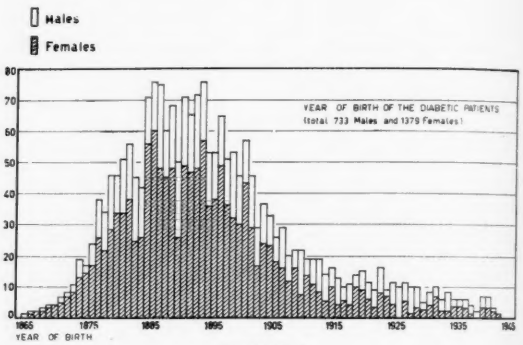


FIGURE 1

Based on data from the English Bureau of Statistics, Pyke demonstrated that with aging a surplus of women develops naturally among the population as a whole.[2] This also holds true for the Netherlands, although not to the same degree as in England (see table 1). The data for the Netherlands for 1953 were provided by the Dutch Central Bureau of Statistics (The Hague).

TABLE 1

Number of females per 100 males

| Age group | England Pyke | The Netherlands | Diabetics Diabetes Clinic Groningen absolute figures |
|---|---|---|---|
| 0-14 | | 95 | |
| 15-19 | | 96 | 63 (16 males— 10 females) |
| 20-29 | | 101 | 74 (46 " — 34 " ) |
| 30-39 | | 102 | 95 (60 " — 57 " ) |
| 40-49 | 100 (45-49) | 105 | 114 (90 " —103 " ) |
| 50-59 | 110 | 105 | 214 (141 " —302 " ) |
| 60-69 | 131 | 107 | 222 (199 " —442 " ) |
| 70-79 | 149 (and older) | 119 | 292 (26 " — 76 " ) |

From the Clinic for Internal Diseases of the State University of Groningen, Netherlands. Director: F. S. P. van Buchem, M.D.

Table 1 shows that the predominance of female diabetics in the groups fifty years of age and older can certainly not be explained from the natural surplus. (The deviations with regard to the proportional figures in the whole Dutch population are not significant for the groups below age fifty (at 5 per cent level), but they are very significant higher than age fifty). The causes usually mentioned are that more women are obese, that they have a less active way of living, and that the menopause may be of influence (Joslin et al.).[4] We shall revert to this later.

Pyke[2] believed that he demonstrated a relationship between pregnancies and incidence of diabetes mellitus. The chance of developing diabetes would appear greater with increasing parity. We investigated this relationship in a group of 299 married women in whom diabetes had developed after forty-five years of age (table 2). This table gives a comparison of the percentages of the 299 diabetic subjects with various numbers of children, with the corresponding percentages of the Dutch women as a whole in 1947 and who were married before 1918, therefore we consider these comparable groups. As in Pyke's investigation, only the live births are included in the table. Unfortunately the number of stillborn is unknown in both groups. Our data were provided by the Central Bureau of Statistics (The Hague).

TABLE 2

| Number of children | Normal 423,797= 100 per cent | Married women diabetics 299=100 per cent | absolute figures |
|---|---|---|---|
| 0 | 8.12 | 7.69 | 23 |
| 1 | 8.45 | 8.03 | 24 |
| 2 | 14.98 | 17.39 | 52 |
| 3 | 14.14 | 12.37 | 37 |
| 4 | 11.78 | 14.38 | 43 |
| 5 | 9.42 | 8.70 | 26 |
| 6 | 7.63 | 7.36 | 22 |
| 7 | 6.19 | 6.69 | 20 |
| 8 | 5.17 | 4.68 | 14 |
| 9 | 4.15 | 6.35 | 19 |
| 10 or more | 9.98 | 6.35 | 19 |

Table 2 shows at a glance that there is no correlation between the incidence of diabetes and the number of children. This impression was confirmed by calculating chi-square at 5 per cent level.

Figure 2 gives a survey of the duration of diabetes from the time of diagnosis in 716 males and 1,331 females. Most of the patients had had diabetes for less than ten years. This can partially be explained from the marked rise in the number of diabetic patients who have visited the outpatient clinic since 1946. The steady yearly increase of the number of patients beginning in 1920, was interrupted during the years 1940-45, especially during 1943-45. This was probably due to World War II, food being scarce especially from 1943 to 1945, as it is known that a limited supply of calories exerts a favorable influence on the pathogenesis and severity of diabetes; this was noticed by Bouchardat as early as 1870 during the siege of Paris.

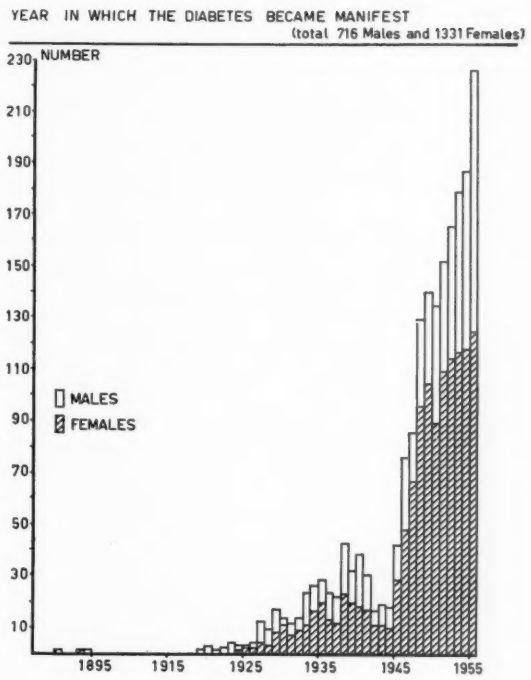


FIGURE 2

The correlation between use of insulin, age and year of onset of the diabetes, as determined for the men, is shown in figure 3. This indicates that most of the older patients can be regulated with diet only, especially during the first five years. Then there is a gradual rise of the quantity of insulin required, which is, however, usually moderate. Only a few of the younger patients are able to get along on a controlling diet alone; most need insulin from the beginning with the daily requirement rising rapidly as a rule.

According to Labbé, a distinction may be made between "diabète maigre" and "diabète gras." The latter form of diabetes is attended by obesity and occurs especially in adults over thirty years of age. Satisfactory regulation of the diabetes is usually obtained with a low-calorie diet, and then the carbohydrate tolerance generally improves. These patients usually show a striking insensitivity to insulin and diabetic coma occurs only rarely in them. The "diabète maigre" is attended by a

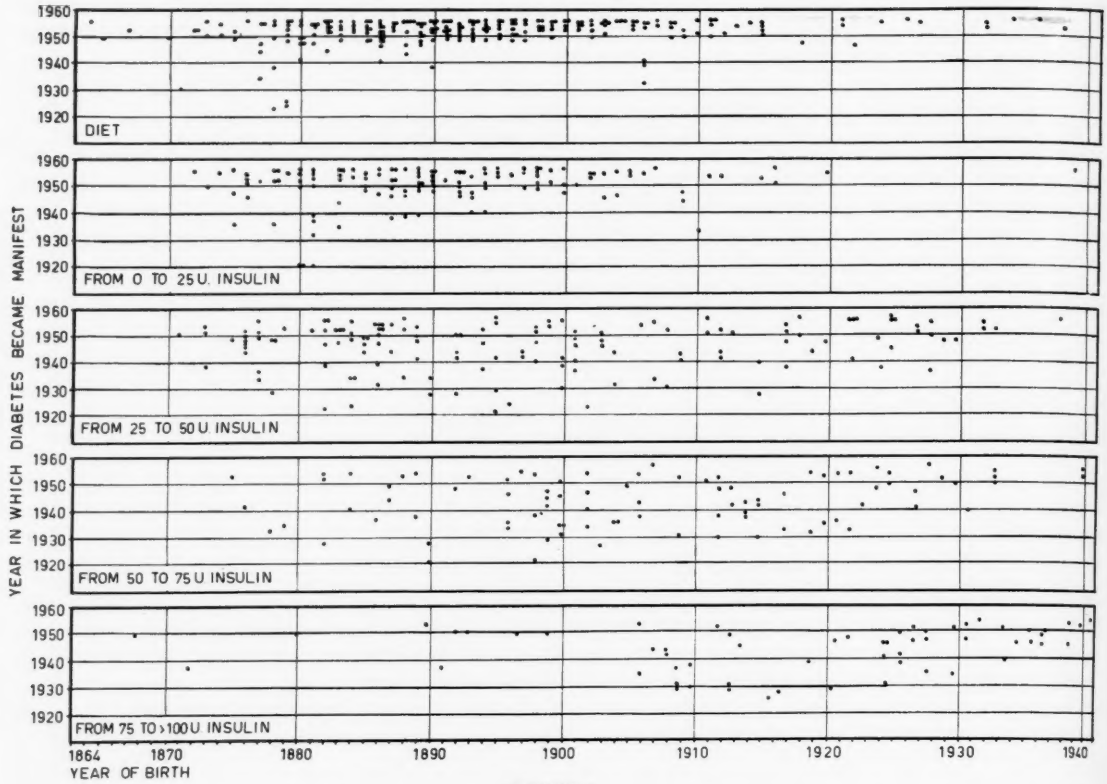


FIGURE 3

low body weight and occurs more frequently in the younger patients. These patients have to be treated with insulin and they react well to it. They have a far greater tendency to develop diabetic coma.

In "diabète gras" there would appear to be rather decreased sensitivity to insulin, in contrast to "diabète maigre" in which a deficiency of endogenous insulin is said to exist. This theory is supported by the findings of Bornstein.[6] In fifteen patients of the latter category he was unable to demonstrate insulin in the plasma, while he found it in thirteen patients with obesity without acidosis (0.10-0.32 mU/ml.). In normal people he found 0.24-0.40 mU/ml.

Our patients of whom height, body weight and age were recorded in our clinic at the beginning of the disease, are divided into three groups according to weight: overweight (D), with normal weight (N) and underweight (M). This division is not easy because we have insufficient data for normal weight in the Dutch population by age, height and sex.

We used the tables given by Duncan,[5] which agree roughly with those of Pett and Ogilvie (in Brozek).[5] Thin persons under forty-five years were considered those weighing less than the average by 3 kg. or more, as indicated for the given height, sex and age. The overweight weighed 3 kg. or more above the average. For patients over forty-five the range of variation of 5 kg. above or below the average was taken as normal, because the "normal" variation seems to be greater. Moreover, if we had taken a smaller range of variation, the figures would be even more favorable to our conclusions.

Accordingly the data of 482 men and 921 women were collected and represented in figure 4. It appears that "diabète gras" is especially a disease of the females (57.11 per cent) and that it occurs far less frequently in males (21.58 per cent). It therefore seems probable that the predominance of the female diabetics is especially connected with the obesity. We would have more certainty about this if the weights of males and females of the whole population, subdivided in the same way, were known to us.

Figure 5 shows the age of onset of diabetes for men and women, classified according to "diabète maigre" and "diabète gras." "Diabète gras" as a rule does not appear

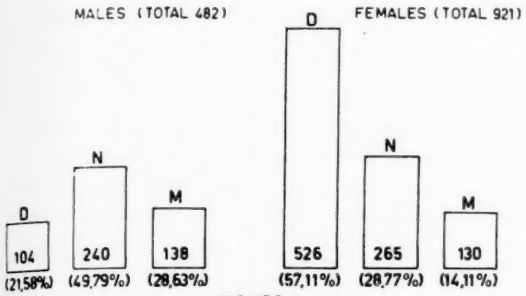


FIGURE 4

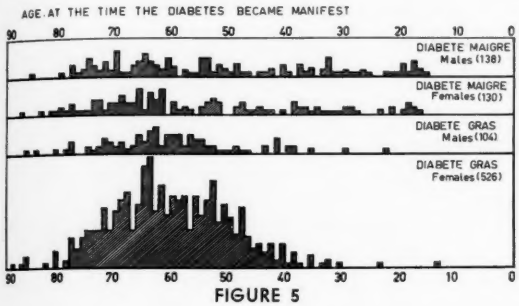
AGE AT THE TIME THE DIABETES BECAME MANIFEST
90 80 70 60 50 40 30 20 10 0
DIABETE MAIGRE Males (138)
DIABETE MAIGRE Females (130)
DIABETE GRAS Males (104)
DIABETE GRAS Females (526)

FIGURE 5

until the thirtieth year of life, in contrast to "diabète maigre," which may occur in youth as well as in older persons. This holds true for both men and women. The young diabetics therefore usually belong to the "diabète maigre" group, while both forms occur among the older patients.

The relationship between age of onset of diabetes and the use of insulin on Jan. 1, 1956, was studied both for "diabète maigre" and diabète gras." Figures 6 and 7 show that the curve for "diabète maigre" presents an identical pattern for men and women. Here also it is true that diabetes with onset at early age requires more insulin than when it develops at more advanced age. The possibility must, however, be kept in mind that among the patients who developed the disease at early age there may have been several who had been under treatment for a long time, so that they needed more insulin. In patients with onset in adult life, dietary treatment, combined or not combined with moderate quantities of insulin, is often sufficient. In the large majority of "diabète gras" cases dietary treatment is sufficient. As defined before, the diets are calculated to provide minimal glycosuria, to reduce the blood sugar content to values below 200 mg. per 100 ml., and, in the case of overweight diabetic patients, the total caloric allotment is restricted to about 1,200 calories, to produce loss of weight.

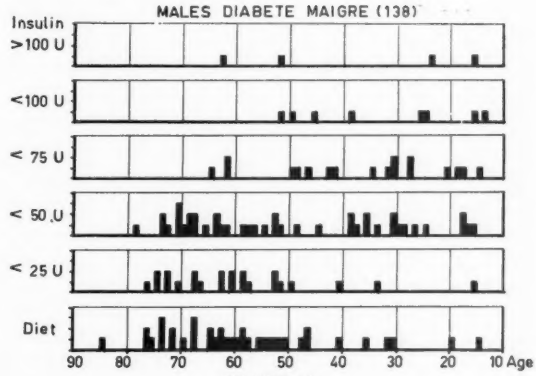


FIGURE 6A

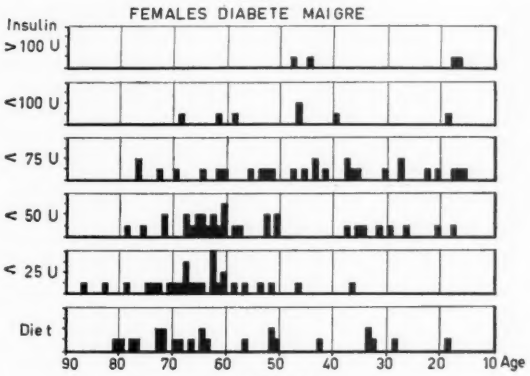


FIGURE 6B

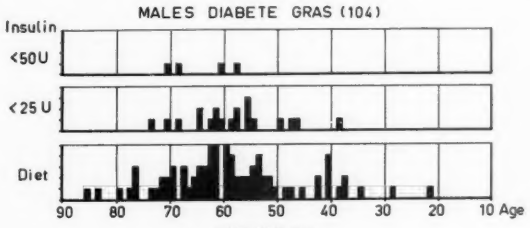


FIGURE 7A

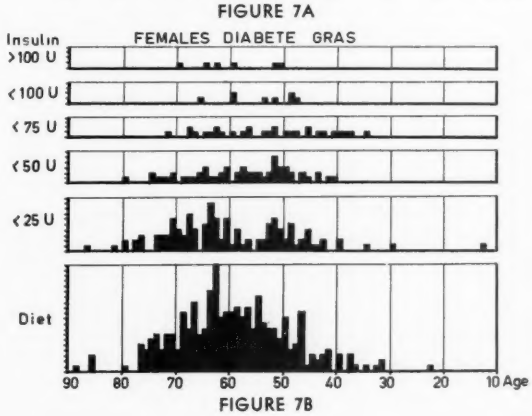


FIGURE 7B

Among the overweight women there were a number who were given higher doses of insulin ($> 50$ U); such cases are not found among the men. It appeared that twenty of these women did not follow the instruction to restrict the caloric intake and their weight increased. The result of treatment with high doses of insulin in these cases was also poor. The weight of eleven of these patients was reduced but in most cases not to normal values. With high doses of insulin the regulation was satisfactory. In seven cases the weight did not change, and with insulin it was possible to obtain a satisfactory result.

Of 484 women and 100 men with "diabète gras" the body weight was known both at the time of onset of the diabetes and on January 1, 1956. These patients are subdivided into the following four groups, according to the change in weight: Group I: The body weight fell to normal values; Group II: the body weight decreased but is still too high; Group III: the weight has remained the same (i.e., changed less than 1 kg.); Group IV: the weight has increased.

TABLE 3

| | Males (100) | per cent | Females (484) | per cent |
|---|---|---|---|---|
| Group I | 44 | 44 | 122 | 25 |
| Group II | 41 | 41 | 217 | 45 |
| Group III | 7 | 7 | 47 | 10 |
| Group IV | 8 | 8 | 98 | 20 |

This table shows that in proportion there was far less loss of weight among women during treatment than among men. From this it is clear that reduction of weight is not only of great importance for the prevention of manifest diabetes, but also that reduction of weight is necessary to reach a balanced carbohydrate metabolism in obese diabetic patients.

## SUMMARY

Data of 733 male and 1,379 female diabetic patients have been evaluated. It is shown that female predominance is especially manifest in the fifty years of age and over group.

No relationship was established between the number of pregnancies and pathogenesis of diabetes.

"Diabète maigre" was found in young and older patients; "diabète gras" only at ages over thirty. The incidence of "diabète gras" cases was far higher among women (57 per cent) than among men (22 per cent). Findings among women indicate that obesity is a stimulating factor in the pathogenesis of diabetes mellitus and that the high frequency of diabetes among women over fifty is associated with obesity. The unfavorable influence of obesity on diabetes is indicated by the higher insulin requirement among overweight women, who showed insufficient loss of weight during the treatment.

## SUMMARIO IN INTERLINGUA

*Investigationes Statistic In Diabete Mellite*

Es evalutate datos ab 733 masculos e 1.379 femininas diabetic. Es monstrate que le predominantia feminin es specialmente manifeste in personas de plus que cinquanta annos de etate.

Esseva establite nulle relation inter le numero de pregnantias del diabeticas e le pathogenese de lor morbo.

*Diabete maigre* esseva trovate in juvene patientes e in patientes de etates plus avantiate. *Diabete gras* occurreva solmente a etates de plus que trenta annos. Le incidentia de *diabete gras* esseva multo plus alte in femininas que in masculos (57 e 22 pro cento, respectivemente). Le datos pertinente al feminas supporta le idea que obesitate es un factor stimulatori in le pathogenese de diabete mellite e que le alte frequentia de diabete mellite in femininas de plus que cinquanta annos de etate es associate con le presentia de obesitate. Le effecto disfavorabile de obesitate super le diabete es reflectite in le plus alte requirimentos de insulina inter femininas con excessos de peso e inadequate perditas de peso sub le effecto del tractamento.

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# Carbohydrate Metabolism and Insulin Sensitivity in the Adrenalectomized Dog

## Studies with $C^{14}$ Glucose

*N. Altszuler, Ph.D., R. Steele, Ph.D., A. Dunn, Ph.D., J. S. Wall, Ph.D., and R. C. de Bodo, M.D., with the technical assistance of C. Bjerknes, G. Durandy and S. Ambrus, New York City*

It is well established that adrenalectomy results in disturbances in carbohydrate metabolism.[1,2] One indication of this disturbance is the greater than normal sensitivity of the adrenalectomized animal to the hypoglycemic effect of insulin.[3-5] However, the adrenalectomized animal is less sensitive in this respect than the hypophysectomized one.[6,7] Thus small doses of insulin (0.025 U/kg.), which were shown to produce severe hypoglycemia in the hypophysectomized dog, produced in nine out of twelve adrenalectomized dogs studied, a fall in the blood glucose concentration similar to that obtained in normal animals. In the remainder the insulin effect was greater than in normal animals, but, even so, was less than in the hypophysectomized animals.

In order to gain insight into the metabolic disturbances responsible for this difference in insulin sensitivity between hypophysectomized and adrenalectomized animals, studies using a $C^{14}$-glucose dilution technic[8] were carried out in these animals. This technic, which utilizes the intravenous administration of trace amounts of $C^{14}$-labeled glucose to unanesthetized animals, measures the size of the body glucose pool and its rate of turnover. Studies of this kind have already been reported for the normal and hypophysectomized dog.[9]

The present study, using the above technic, is concerned with the following aspects of carbohydrate metabolism in the adrenalectomized dog: (a) size of the glucose pool, (b) rate of glucose inflow from the liver into the plasma, i.e., glucose production, and (c) rate of glucose outflow from the plasma into the tissues, i.e., glucose utilization. The present study also considers the changes in the rates of glucose production and utilization following an intravenous injection of insulin: (a) during the development of hypoglycemia, and (b) during the period of restoration of plasma glucose concentration.

Isotopically-labeled glucose has been used to measure the size and turnover rate of the body glucose pool in the rat,[10-11] dog[12-14] and man.[15] In the $C^{14}$-glucose dilution technic employed in the present studies, uniformly labeled $C^{14}$ glucose was administered intravenously by a single "priming" injection along with a constant continuous infusion. This combination secured a relatively unchanging circulating glucose specific activity for several hours. The continuous infusion of $C^{14}$ glucose (which is given in virtually weightless amount, i.e., only a few milligrams during a whole experiment) balances the endogenous $C^{12}$ output by the liver so that the combined inflow of glucose has about the same $C^{14}$-glucose specific activity as that which prevails in the circulating glucose as a result of the "priming" injection. The course pursued by the plasma glucose specific activity after the $C^{14}$-glucose infusion is started provides a measure of the size of the body glucose pool (in grams of glucose) and the rate of its turnover. In the postabsorptive period, when the blood glucose level remains constant, and no glucose is excreted in the urine, glucose production and utilization are equal and the glucose pool is in a "steady state." Under these conditions the term "turnover" refers to glucose utilization and production.

When insulin is injected during the period of continuous infusion of $C^{14}$ glucose, the specific activity of the blood glucose is not influenced by the resulting increase in the rate of glucose utilization; it is altered only by changes in the rate of production of endogenous $C^{12}$ glucose by the liver. Thus following insulin injection the rate of glucose production during a time interval

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between blood samples is measured by the difference in specific activities between the two samples. To measure the rate of glucose utilization during the same time interval, one takes into account the rate of glucose production and the difference in the glucose concentration between the two samples. A detailed description of the method of calculation of glucose production and utilization in the steady state[8] and following insulin[16] has been given elsewhere.

## METHODS

Adult-trained, unanesthetized, adrenalectomized dogs, three to thirty months postoperative, were used. The animals were fed a standard diet[17] and maintained on daily minimal doses of desoxycorticosterone acetate, 2.5 mg./kg. (Cortate, Schering). The animals were used seventeen to eighteen hours after the last food intake. A detailed description of the procedure and equipment has been given previously.[8]

Uniformly labeled $C^{14}$ glucose was administered intravenously by a priming injection, followed immediately by a constant infusion continued throughout the entire experiment. Plasma glucose concentrations and specific activities were determined using blood samples withdrawn at intervals from the exposed femoral vein. Samples were collected during the 60-120 min. period of $C^{14}$-glucose infusion to establish a base line with which to compare findings subsequent to insulin injection. At 120 min., glucagon-free insulin (Lilly), dissolved in saline adjusted to *p*H 3 with hydrochloric acid, was injected into the saphenous vein. Blood samples were collected more frequently just after the administration of insulin and again at longer intervals of time until three hours had elapsed following insulin injection. The plasma glucose content was determined on aliquots of Somogyi zinc-barium filtrates[18] by the method of Hagedorn-Jensen.[19] The preparation of the glucose in each filtrate for counting the radioactivity has been reported previously.[8,20] The rates of glucose production and utilization prior to and following insulin injection were calculated as before.[8,16]

## RESULTS

The difference in degree of insulin hypersensitivity between the adrenalectomized and hypophysectomized animal is evident from figure 1, which presents typical curves for the blood glucose concentration following the intravenous injection of insulin (0.025 U/kg.) into normal and hypophysectomized dogs and an adrenalectomized dog. It should be noted that despite the low serum sodium values in the adrenalectomized animal due

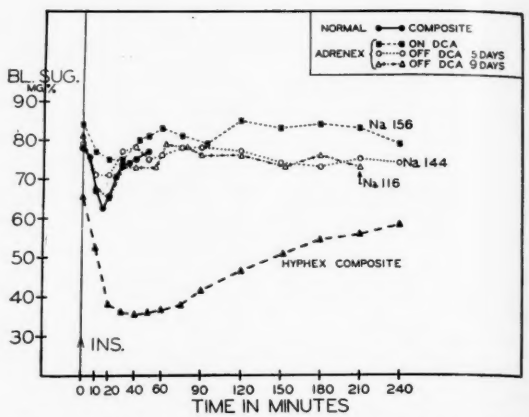


**FIG. 1. Blood sugar curves following intravenous injection of insulin (0.025 U/kg.) into an adrenalectomized (adrenex) dog compared with those obtained in normal and hypophysectomized (hyphex) dogs. The curves for the adrenalectomized animal were obtained while the animal was on the usual DOCA maintenance (2.5 mg./day) and again while off DOCA. (Reprinted with permission from "Recent Progress in Hormone Research" Vol. VIII: 511, 1953. New York, Academic Press, Inc.)**

to discontinuation of DOCA maintenance, the sensitivity to the small dose of insulin is not increased. In a majority of the adrenalectomized dogs (75 per cent of the dogs studied), this small dose of insulin produces glycemic curves similar to those in the normal, in that the extent of the hypoglycemia and the period of restoration of the blood sugar concentration to control levels are similar in both types of animals. This is in contrast to the severe hypoglycemia and prolonged recovery period of the blood glucose concentration observed in the hypophysectomized dog. Some adrenalectomized animals exhibit a greater sensitivity to this dose of insulin than do normal animals, but the severity of the hypoglycemia and extent of the recovery period are never as marked as in the hypophysectomized animal. Figure 2 demonstrates that removal of the pituitary gland in adrenalectomized animals results in a greatly increased sensitivity to the hypoglycemic effect of insulin, thus indicating that the pituitary gland in the adrenalectomized animal still exerts an effect on carbohydrate metabolism.

Table 1 presents data obtained in experiments wherein the circulating glucose was tagged with $C^{14}$. It is evident that the adrenalectomized animal in the postabsorptive state has a higher plasma glucose concentration than the hypophysectomized animal. The body glucose pool is likewise proportionately greater in the adrenalectomized animal. It can be seen that the rate of glucose inflow and outflow (turnover, production and utilization) in the adrenalectomized dog is somewhat below

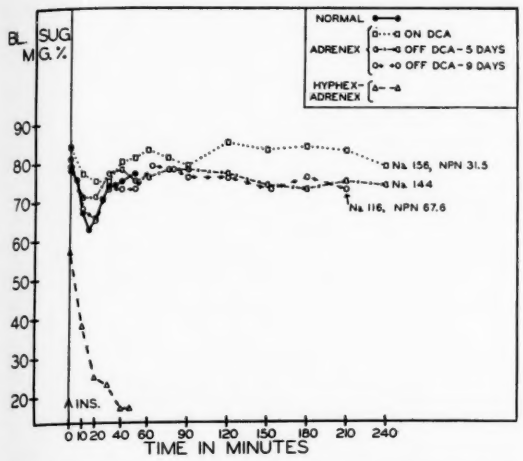


FIG. 2. Blood sugar curves following intravenous injection of insulin (0.025 U/kg.) into an adrenalectomized (adrenex) dog compared with those obtained in normal and hypophysectomized-adrenalectomized (hyphex-adrenex) dogs. The curves for the adrenalectomized and hypophysectomized-adrenalectomized dogs were obtained while both were on DOCA maintenance (2.5 mg./day) and also while the former was off DOCA. (Reprinted with permission from "Annals of the New York Academy of Sciences," Vol. 57: 23, 1953.)

TABLE 1

Effect of adrenalectomy and hypophysectomy on plasma glucose concentration, glucose pool, glucose space and rates of glucose inflow from the liver into plasma and outflow from plasma into tissues.

| Dog status | Average venous plasma glucose mg. per cent | Glucose pool mg./kg. | Glucose space per cent B. wt. | Glucose inflow and outflow g/m²/hour |
|---|---|---|---|---|
| Normal (13)* | 108 | 275±11† | 26±1 | 3.85±0.16 |
| Adrenalectomized‡ (6) | 98 | 247±19 | 25±1 | 3.46±0.11 |
| Hypophysectomized (8) | 89 | 192±16 | 22±2 | 2.63±0.23 |

*Number of experiments.
†Mean and standard error of mean.
‡Maintained on DOCA 2.5 mg./day.

normal, but still higher than that in the hypophysectomized dog.

Intravenous injection of insulin into these animals results in an increase in the rate of glucose outflow from the plasma, as shown in figure 3. The clear part of the bar represents the rate of glucose utilization (outflow) in the twenty minutes prior to insulin injection. Since this period of time still represents the steady state, this value for utilization equals the rate of glucose production by the liver. The hatched part of the bar indicates the increase in glucose utilization in the twenty minutes following insulin injection. The entire height of the bar thus represents the total glucose utilization following insulin injection. The twenty-minute period was selected because it represents the period of maximum effect of this dose of insulin on glucose utilization. It is evident from figure 3 that the increase in glucose utilization following injection of insulin, 0.025 U/kg., into the adrenalectomized dog is similar to that produced in the normal one. This is to be contrasted with the much greater increase in utilization found in the hypophysectomized dog, which was reported previously.[21]

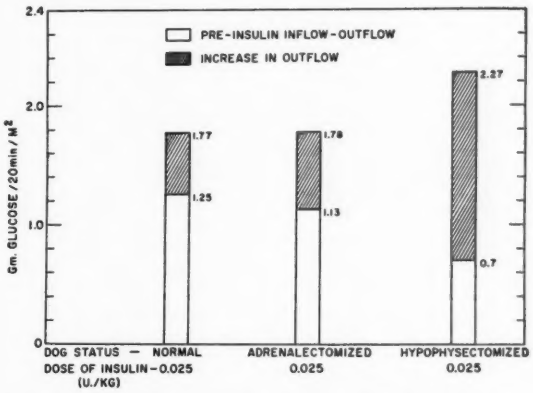


FIG. 3. Composite changes in glucose outflow from body glucose pool into tissues during the first twenty minutes following intravenous injection of insulin (0.025 U/kg.) compared with the pre-insulin glucose inflow-outflow values in normal, adrenalectomized and hypophysectomized dogs. Pre-insulin glucose inflow-outflow (unshaded bar); increase in glucose outflow (hatched bar); total glucose outflow = total height of bar.

The increase in the rate of glucose utilization following insulin injection results in hypoglycemia, as shown in the upper half of figure 4. The hypoglycemia is more severe in the hypophysectomized dog than in the adrenalectomized animal. The response to the insulin-induced hypoglycemia is shown in the bottom half of figure 4. In the normal and adrenalectomized animals the hypoglycemia evokes a substantial increase in glucose inflow into the plasma thereby elevating the blood glucose concentration, whereas in the hypophysectomized animal there is little or no increase in glucose inflow and consequently the hypoglycemia is prolonged.

Although in the adrenalectomized dog the effect of the small dose of insulin (0.025 U/kg.) on glucose utilization and the response to the induced hypoglycemia are similar to that observed in the normal animal, the similarity ends when larger doses of insulin are used. Figure 5 contrasts the effects of the small and larger dose of insulin in the same adrenalectomized dog. The ef-

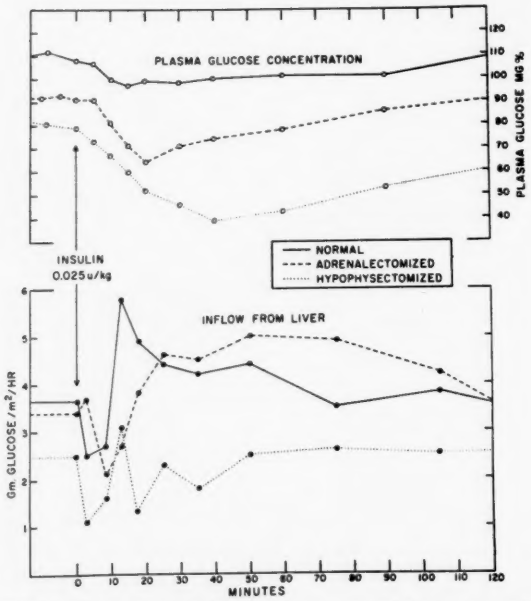


FIG. 4. Comparison of plasma glucose concentration curves and rates of glucose inflow from the liver into the plasma in a typical normal, adrenalectomized and hypophysectomized dog following intravenous injection of insulin (0.025 U/kg.). The values for glucose inflow from the liver prior to zero time are control values for glucose turnover (inflow and outflow) for each dog obtained in the same experiment prior to insulin injection.

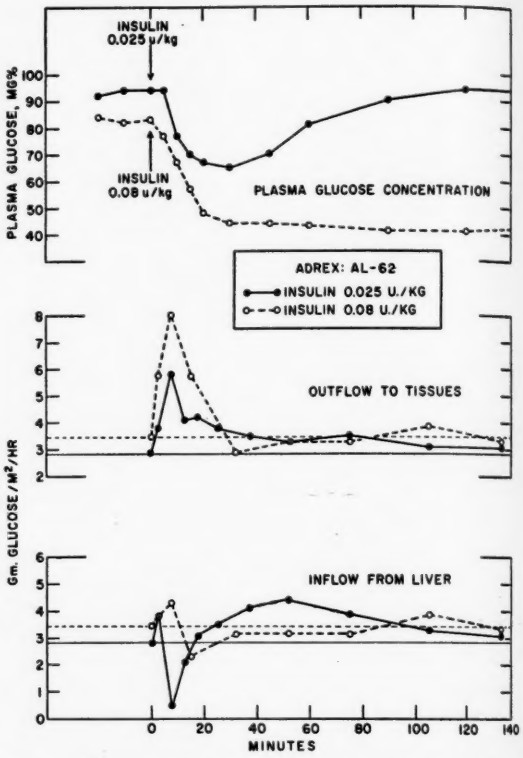


FIG. 5. Changes in the plasma glucose concentration, glucose outflow from plasma and glucose inflow from liver into plasma observed in the same adrenalectomized (adrex) dog, following intravenous injection of two different doses of insulin, 0.025 U/kg. and 0.08 U/kg. Lighter reference lines across graph represent control rates of glucose inflow and outflow obtained in the same experiment prior to insulin injection. The adrenalectomized dog maintained on DOCA (2.5 mg./day).

fect of the 0.025 U/kg. dose is the same as described in figures 3 and 4. The larger dose of insulin, 0.08 U/kg., produces a greater increase in glucose utilization and a more marked hypoglycemia. However, the glucose production in response to the hypoglycemia is not increased and consequently the hypoglycemia is prolonged. Thus the larger dose of insulin is followed by a greater increase in utilization of plasma glucose which evokes little or no change in glucose production. As in the hypophysectomized dog, both of these factors are implicated in the greater-than-normal sensitivity of the adrenalectomized animal to the larger doses of insulin.

## DISCUSSION

The disturbances in carbohydrate metabolism of the adrenalectomized animal appear to be less severe than those of the hypophysectomized animal. This distinction was made in earlier studies by using the insulin sensitivity and glucose tolerance tests. The contrast in the insulin sensitivity between the adrenalectomized and hypophysectomized dogs is shown in figure 1. Likewise it was shown that following an intravenous infusion of a glucose load the blood glucose concentration in the adrenalectomized dog does not fall to the very low levels ("secondary hypoglycemia") which are reached in the hypophysectomized animal.[7]

The present studies further distinguish between the adrenalectomized and hypophysectomized animal. In the adrenalectomized animal, in the undisturbed postabsorptive state, the glucose pool size and the rates of glucose production and utilization are nearly normal and are higher than the respective low values observed in the hypophysectomized animal. Inasmuch as both types of animals are deficient in the 11,17-oxycorticosteroids, it is obvious that these differences are due to the presence of the pituitary gland in the adrenalectomized dog.

The pituitary hormone responsible for this difference is believed to be growth hormone. This has been deduced in earlier studies[22] from the following observations: (a) removal of the gonads in the adrenalectomized dog did not render the animal more sensitive to insulin,

thus arguing against a role of the gonadotropins in this respect; (b) administration of large doses of thyroxine to hypophysectomized dogs resulted in only a slight amelioration of their insulin hypersensitivity, thus mitigating against the importance of thyrotropin as an anti-insulin agent; (c) prolactin administration in the hypophysectomized dog ameliorated the insulin hypersensitivity, but its effect was not as marked as that produced by administration of growth hormone; (d) ACTH, which exerts its influence on carbohydrate metabolism through the adrenal cortex, when administered to the adrenalectomized animal did not alter the insulin sensitivity of the animal. From these observations it was concluded that the difference between the adrenalectomized and hypophysectomized animals in the degree of metabolic disturbance, as evidenced by insulin sensitivity and glucose tolerance tests, was due to the presence of growth hormone in the adrenalectomized animal.

In view of the above observations, it is reasonable to assume that the endogenous growth hormone in the adrenalectomized animal is also responsible for the larger glucose pool size and higher rates of glucose production and utilization than were observed in the hypophysectomized dog. This assumption is further supported by the observation that a growth hormone regimen in the hypophysectomized dog increases its small pool size and markedly increases the low rates of glucose production and utilization.[23,24]

The present studies also suggest, however, that the influence of growth hormone in the adrenalectomized animal is limited. This is evident from the difference in response obtained when the dose of injected insulin was increased. Whereas the hypoglycemia induced by the small dose of insulin (0.025 U/kg.) evoked a rapid increase in glucose production, the larger dose of insulin (0.08 U/kg.) failed to do so (figure 5). The significance of the latter observation remains to be clarified, but it does suggest a limitation in the ability of growth hormone to maintain the normal responsiveness to insulin-hypoglycemia. The limited effectiveness of endogenous growth hormone in the adrenalectomized animal may be due to several factors: (a) the secretion of growth hormone may be decreased, (b) the presence of adrenal steroids may be required for growth hormone to exert its full effect, and (c) the growth hormone secreted under physiological conditions may enhance, but to a limited degree, the ability of the animal to mobilize carbohydrate stores rapidly. At present there is no available evidence that the secretion of growth hormone in the adrenalectomized animal is diminished.[25] There is evidence in support of the second possibility, that growth hormone is more effective in the presence of the adrenal steroids. Thus it was shown that administration of growth-promoting anterior pituitary extract in the adrenalectomized animal resulted in a lesser increase in ketone body formation than in the normal one.[26] Also in the hypophysectomized rat, adrenal steroids had to be administered along with growth hormone to demonstrate the latter's inhibitory influence on the insulin effect in the isolated diaphragm.[27] On the other hand, it is evident that a growth hormone regimen in the hypophysectomized[17] or hypophysectomized-adrenalectomized[28] dog still exerts a marked anti-insulin effect, despite the deficiency or absence of adrenal steroids in these animals.

Regarding the third possibility, it is believed that endogenous growth hormone contributes to the maintenance of the glucose pool size and rates of glucose production and utilization in the undisturbed postabsorptive state. It is also evident that the influence of growth hormone facilitates the increase in glucose production in response to the hypoglycemia induced by the small dose of insulin. However, this influence apparently is limited, inasmuch as the glucose production is not increased substantially following hypoglycemia induced with larger doses of insulin. On the other hand, administration in the adrenalectomized and hypophysectomized animal of adrenal oxycorticosteroids in doses within the physiological range does render them capable of increasing glucose production in response to hypoglycemia induced by small as well as larger doses of insulin.

It would appear, therefore, that in physiological life, growth hormone and the adrenal steroids complement each other in modulating the homeostatic mechanisms which regulate carbohydrate metabolism.

## SUMMARY

1. Using a $C^{14}$-glucose dilution technic, it was found that the unanesthetized adrenalectomized dog, in the postabsorptive state, has a nearly normal glucose pool size and glucose turnover rate, in contrast to the much lower values observed in the hypophysectomized dog.

2. Injection of a small dose of insulin (0.025 U/kg.) into the adrenalectomized dog results in an increase in glucose utilization similar to that produced in the normal dog, and likewise the increase in glucose production in response to the induced hypoglycemia is adequate to elevate the plasma glucose concentration promptly to control levels; in the hypophysectomized animal this dose of insulin results in a greater increase in glucose utilization, and the increase in glucose production is inadequate, thereby resulting in a prolongation of the hypoglycemia.

3. Injection of larger doses of insulin into the adrenalectomized animal brings about a greater increase in glucose utilization, but the resulting hypoglycemia does not evoke an adequate increase in glucose production; consequently the hypoglycemia is prolonged.

4. The aforementioned differences between the hypophysectomized and adrenalectomized animals are attributed to the presence of growth hormone in the latter.

5. The implications of the significance of physiological growth hormone on carbohydrate metabolism are discussed.

## SUMMARIO IN INTERLINGUA

*Metabolismo De Hydrato De Carbon E Sensibilitate Pro Insulina In Adrenalectomisate Canes: Studios Con Glucosa A $C^{14}$*

1. Per medio del technica a dilution de $C^{14}$ intra glucosa, il esseva trovate que le non-anesthesiate adrenalectomisate can ha in le stato postabsorptive un reserva de glucosa de magnitude quasi normal e etiam un quasi normal rapiditate del utilisation e consumption de glucosa, per contrasto con le multo plus basse valores observate in le hypophysectomisate can.

2. Le injection de un micre dose de insulina (0,025 U/kg.) resulta, in le adrenalectomisate can, in un augmento del utilisation de glucosa que es simile a illo producite in le can normal. Etiam, le augmento del production de glucosa in responsa al inducite hypoglycemia suffice pro elevar le concentration de glucosa in le plasma promptemente usque al nivello de controlo. In le hypophysectomisate can, un tal dose de insulina resulta in un plus grande augmento del utilisation de glucosa, durante que le augmento del production de glucosa es inadequate con le consequentia de un prolongate persistentia del stato hypoglycemic.

3. Le injection de plus grande doses de insulina in le adrenalectomisate can effectua un plus grande augmento del utilisation de glucosa, sed le resultante hypoglycemia non evoca un adequate augmento del production de glucosa, de maniera que le hypoglycemia es prolongate.

4. Le supra-mentionate differentias inter le hypophysectomisate e le adrenalectomisate can es attribuite al presentia de hormon de crescentia in le secunde.

5. Es discutite le repercussiones del effecto de physiologic hormon de crescentia super le metabolismo de hydrato de carbon.

## ACKNOWLEDGMENT

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## *Glucagon Effect on Gastric Activity*

Glucagon, known as the pancreatic-hyperglycemic-glycogenolytic factor, HGF, has been the subject of several reviews dealing with its function. T. B. Van Itallie (*New Eng. J. Med. 254*:794, 1956) has reviewed the physiologic and clinical aspects of glucagon and recent work has been summarized in *Nutrition Reviews* (*12*: 132, 1954; *13*:325, 1955; *14*:304, 309, 1956). The discovery of glucagon was made by C. P. Kimball and J. R. Murlin (*J. Biol. Chem. 58*:337, 1923) while determining the efficacy of various solvents and precipitants in the purification of insulin. J. F. Fodden and W. O. Read (*Endocrinology 54*:303, 1954) observed a depletion of the hyperglycemic action of the pancreas by the use of Synthalin A, a material that is toxic to pancreatic alpha cells. This evidence that the alpha cells are the source of glucagon has been supported by the observation of S. A. Bencosme, E. Leipa and S. S. Lazarus (*Proc. Soc. Exp. Biol. & Med. 90*:387, 1955). They showed that extracts of the uncinate process of the dog pancreas, which is devoid of alpha cells, contained no hyperglycemic activity. The mechanism by which glucagon causes a rise in blood sugar is its glycogenolytic action on the liver, according to E. W. Sutherland and C. F. Cori (*J. Biol. Chem. 188*:531, 1951). R. M. Zollinger and E. H. Ellison (*Ann. Surg. 142*:709, 1955) proposed that peptic ulceration associated with gastric hyperacidity, which could not be controlled surgically or medicinally, might be the result of certain pancreatic islet cell tumors producing excessive amounts of glucagon. C. S. Hoar and J. R. Browning (*New Eng. J. Med. 255*:153, 1956) demonstrated that decreases in blood pepsinogen levels correlate directly with induced decreases in gastric peptic activity . . .

A. J. Stunkard, T. B. Van Itallie and B. B. Reis (*Proc. Soc. Exp. Biol. & Med. 89*:258, 1955) noted that injection of glucagon in human volunteers abolished hunger contractions. In contradistinction to the experiments of Zollinger and Ellison, these findings suggest that glucagon, rather than stimulating it, depresses gastric secretory activity. Further study is needed to determine the identity and define the role of the pancreatic ulcerogenic factor. Patients with intractable ulcers should be studied carefully for evidence of islet cell tumors. The histological and histochemical characteristics of any pancreatic tumors associated with peptic ulceration should be evaluated carefully.

From *Nutrition Reviews*, Vol. 16, No. 2, pp. 60-62, February 1958.

# Studies of 6-Deoxy-6-fluoroglucose and Insulin

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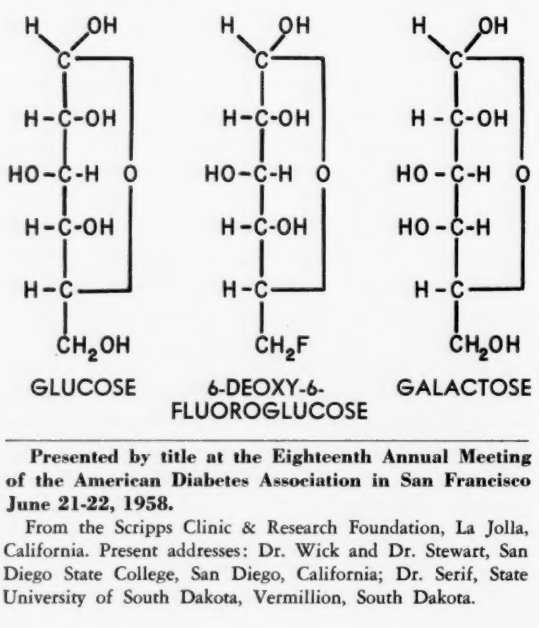
The most widely held hypothesis at the present time regarding the specific site of insulin action in muscle tissue is that insulin is concerned with the intracellular transfer of glucose and that the transfer mechanism is independent of the hexokinase reaction. This conclusion is supported chiefly from studies using galactose,[1,2] a sugar closely related to glucose but one which is not phosphorylated by either brain or yeast hexokinase, except under special conditions.[3] The use of glucose-like compounds for carbohydrate and insulin studies is not always rewarding because one cannot predict in advance whether or not a particular compound will be useful. Most of the compounds examined have been of little value because of their inertness for one reason or another. 6-Deoxy-6-fluoroglucose (6-FG), however, is a compound with interesting physiological properties. Its chemical structure is shown in figure 1. In contrast to galactose, this sugar has all of the stereochemical configuration of glucose and yet cannot be phosphorylated in the number six position. Since spatial configuration of the sugars has been suggested as an important feature of their response to insulin,[4] this compound, therefore, may be an ideal sugar to use in studies related to insulin action and carbohydrate metabolism.




**Presented by title at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco June 21-22, 1958.**

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We have previously reported[5] that 6-deoxy-6-fluoroglucose inhibits the oxidation of uniformly labeled glucose $C^{14}$ in rat kidney slices and that one area of inhibition is prior to the formation of glucose-6-phosphate. These studies have been extended to rat diaphragm and rat adipose (epididymal) tissue in vitro with similar conclusions.[6]

In this report we have examined the insulin responsiveness of this fluorinated sugar using rat diaphragm in vitro and the eviscerated-nephrectomized animal as the test preparations.

## METHODS

*Procedure used with the eviscerated animal*: Nonfasted rabbits were eviscerated-nephrectomized.[7] When radioactive substrates were to be injected, a tracheal cannula was inserted at the time of the operation in order to collect $CO_2$ for $C^{14}$ determinations. The animals were maintained at a normal blood sugar level by the constant infusion of glucose. The glucose utilization values were calculated from the amount of glucose infused each hour. In the experiments in which the effect of 6-FG on glucose disappearance was investigated, the animals were followed for a three-hour period before the injection of the sugar analog and the infusion of glucose was then continued for another three-hour period without interruption. By this procedure each animal acts as its own control. The plasma glucose concentration was determined by the glucose oxidase method. When $C^{14}$ 6-FG was used for volume of distribution studies, plasma samples were taken each hour after the injection of the 6-FG for determination of their $C^{14}$ content. The volume of distribution was calculated from the counts administered and from the counts recovered in the plasma

samples. Insulin,* when administered, was given in maximal amounts.

*Diaphragm experiments*: Nonfasted rats (Sprague-Dawley) weighing approximately 125 gm. were decapitated and exsanguinated. Previously described procedures[8,9] were employed for the preparation of the rat diaphragms and for the measurement of respired $C^{14}O_2$ and uptake of radioactive sugars.

*Preparation of 6-deoxy-6-fluoroglucose*: 6-FG was prepared according to the procedure of Helferich and associates[10,11] as revised by Blakley.[12] When preparing the $C^{14}$-labeled compound, glucose uniformly labeled with $C^{14}$ was used as the starting material.

## RESULTS

*Effect of insulin on uptake of $C^{14}$-labeled 6-FG by rat diaphragm (table 1)*: From the data presented in table 1, it is apparent that the amount of $C^{14}$-labeled 6-FG that entered the diaphragm was not altered appreciably by the addition of insulin or glucose to the incubating media. From these figures, the volume of distribution of 6-FG in the diaphragm appears to be roughly 80 per cent, a figure that is close to total tissue water.

TABLE 1

The effect of insulin on the uptake of 6-deoxy-6-fluoroglucose using isolated rat diaphragm tissues

| No. of experiments | 6-FG uptake (mg./gm. tissue) No insulin | Insulin |
|---|---|---|
| 10 | 1.45 ± 0.52 | 1.47 ± 0.72 |
| 5 | 1.51 ± 0.14* | 1.47 ± 0.60* |

Each flask contained $C^{14}$ uniformly labeled 6-deoxy-6-fluoroglucose (0.01 *M*), approximately 150 mg. (wet weight) diaphragm, insulin (0.50 units) when added, and Krebs-Ringer phosphate buffer (*p*H 7.4) to a total volume of 2 ml. Flasks were incubated at 37° for two hours with $O_2$ as the gas phase. 0.20 ml. *5 N* NaOH and a small square of filter paper were placed in the center well to trap $CO_2$.
*Incubation media also contained 0.01 *M* glucose.

*Effect of insulin on volume of distribution of 6-FG in eviscerated-nephrectomized animals (table 2)*. It is apparent from the data presented here that 6-FG rapidly enters the cells of the extrahepatic tissues and that the administration of insulin does not appear to increase the rate of cell entry or alter the final body distribution values. The total body water of this preparation is approximately 65 per cent of the body weight. In order to eliminate the possibility that the fluorinated sugar was concentrated in some special tissue, an aliquot of muscle tissue (experiment 10) was examined for its $C^{14}$ content at the termination of the experiment. The $C^{14}$ content of this tissue sample was of the order expected from the $C^{14}$ content of the terminal plasma sample. No $C^{14}$ was found in the expired $CO_2$ in these experiments. It is therefore assumed that the disappearance of 6-FG from the circulating plasma is the direct result of cell entry of this compound. The possibility of cell wall adsorption appears to be overruled by the observation that the volume of distribution approaches, but does not exceed, the total body water.

TABLE 2

Apparent volume of distribution of 6-deoxy-6-fluoroglucose in control and in insulin treated eviscerated-nephrectomized rabbits. Values expressed as percentage of body weight.*

| | No insulin | | | | | Insulin treated | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Exp. number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 6-FG injected (mg./kilo) | 33 | 33 | 80 | 200 | 200 | 33 | 33 | 80 | 200 | 200 |
| Time in hours | | | | | | | | | | |
| 1 | 30 | 38 | 29 | 39 | 46 | 35 | 38 | 24 | 47 | |
| 2 | 35 | 42 | 36 | 45 | 46 | 44 | 51 | 27 | 59 | 46 |
| 3 | 38 | 49 | 39 | 47 | 55 | 48 | 52 | 29 | 65 | 49 |
| 4 | 41 | 52 | 38 | 50 | 50 | 52 | 55 | 35 | 61 | 53 |
| 5 | | 49 | | 48 | 55 | 54 | 56 | | 66 | 55 |

*In this preparation the volume of distribution of non-insulin responsive sugars (sorbitol, mannitol, 3-0-methylglucose, etc.) is 25 to 30 per cent of the body weight.

*Effect of 6-FG on glucose utilization (table 3)*: The data in this table show that 6-FG effectively inhibits the intracellular transfer of glucose in the eviscerated animal and that the inhibition is related to the amount of 6-FG injected.

TABLE 3

The effect of a single injection of 6-deoxy-6-fluoroglucose on plasma glucose disappearance rate in eviscerated-nephrectomized rabbits maintained with maximal insulin

| Exp. number | 6-FG injected (mg./kilo) | Glucose disappearance* (mg./kilo/hr.) Before 6-FG | After 6-FG | Per cent decrease |
|---|---|---|---|---|
| 11 | 25 | 378 | *334 | 12 |
| 12 | 50 | 351 | *275 | 22 |
| 13 | 100 | 307 | *230 | 25 |
| 14 | 200 | 388 | *225 | 42 |

*The glucose disappearance values represent a three-hour average before and a three-hour average after the administration of the 6-deoxy-6-fluoroglucose.

## DISCUSSION

The results from this study with 6-FG have revealed the following interesting findings: (1) the compound rapidly enters the muscle cells of the extrahepatic tissues; (2) the compound inhibits the plasma glucose disappearance; (3) the compound does not appear to be insulin responsive.

* The crystalline zinc insulin used was contributed by Lilly Research Laboratories.

Since the data reported here do not provide information regarding the site (or sites) of action of 6-FG with respect to its inhibiting action on glucose transfer, no discussion of this will be attempted in this report. The possible sites of action are discussed in the other papers on this subject. However, it is pertinent that the inhibiting action of 6-FG under the in vivo conditions used here confirms the in vitro findings with kidney slices,[5] rat diaphragm and adipose tissue,[6] and those of Blakley and Boyer[13] with yeast cells.

Perhaps the most significant findings in these studies are that the rate of cell entry of 6-FG is not accelerated by the administration of insulin although the compound can easily cross the muscle cell barrier. It has been our experience (with one exception) when testing hexoses that two conditions must be fulfilled for a compound to be insulin responsive in the living animal (eviscerated). The first of these is that the compound must be capable of phosphorylation by the enzyme hexokinase and second that its Michaelis constant* must be fairly close (enzymatically speaking) to that of glucose ($K_m = 8 \times 10^{-6}$) with which it must compete for cell entry. The hexose which does not appear to meet the above requirements is galactose. This sugar is insulin responsive, although its Michaelis constant ($K_m$ 0.1) is such that it should not be insulin responsive when the same line of reasoning is used as for the other hexoses. Galactose, however, is a novel sugar. It has been definitely established that this compound is not as inert as was earlier believed. For example, galactose may be phosphorylated by yeast hexokinase and oxidized to $CO_2$ by muscle preparations.[2] In view of these observations, it is obvious that the intracellular transfer of galactose must be studied more intensively before its exact role is known with respect to insulin action. The compounds other than glucose that are insulin responsive and their Michaelis constants are: mannose ($K_m = 5 \times 10^{-6}$), 2-deoxyglucose ($K_m = 2.7 \times 10^{-5}$), and glucosamine ($K_m = 8 \times 10^{-5}$). Fructose is insulin responsive when tested in the absence of glucose (rat diaphragm)[14] but in the presence of glucose it cannot compete with glucose[9,15,16] for either cell entry or insulin action, which can be explained on the basis of its unfavorable Michaelis constant ($K_m = 1.6 \times 10^{-3}$).

The inability of insulin to alter the rate of cell entry of 6-FG, which is a compound having the same stereoconfiguration as glucose but incapable of phosphorylation by the enzyme hexokinase, is consistent with the concept that the hexokinase reaction is associated in some way with insulin action in accelerating the cell entry of sugars.

*The Michaelis constant is that concentration of substrate (moles per liter) which allows the enzymatic reaction to proceed at 50 per cent of maximum velocity. This constant is a relative expression (measure) of the affinity of an enzyme for the substrate. Glucose has an approximate 200-fold greater affinity for hexokinase than fructose, i.e., $K_m$ fructose/$K_m$ glucose = 200; whereas mannose has approximately the same affinity as glucose, and 2-deoxyglucose and glucosamine have about one third and one tenth the affinity, respectively, of glucose for hexokinase. The Michaelis constants ($K_m$) listed here are those of Sols and Crane[3] for brain hexokinase. Comparable values for muscle hexokinase are not available.


## SUMMARY

6-Deoxy-6-fluoroglucose (6-FG), a glucose analog which inhibits the oxidation of glucose in kidney slices, has been examined with respect to the action of insulin using rat diaphragm in vitro and the eviscerated-nephrectomized animal. It was found that 6-FG rapidly enters the cells of the extrahepatic tissues and simultaneously inhibits the intracellular transfer of glucose. However, the rate of cell entry of 6-FG or its distribution in tissue water does not appear to be altered by the administration of insulin. Likewise, the uptake of 6-FG by rat diaphragm tissue in vitro is not increased by the presence of insulin in the incubation medium. The significance of these results with respect to the nature of the action of insulin is discussed.

## SUMMARIO IN INTERLINGUA

*Studios Con 6-Disoxy-6-Fluoroglucosa E Insulina*

6-Disoxy-6-fluoroglucosa (6-FG), un analogo de glucosa que inhibi le oxydation de glucosa in trenchos de ren, esseva examinate con respecto al activitate de insulina in diaphragma de ratto in vitro e le eviscerate nephrectomisate animal vive. Esseva trovate que 6-FG entra rapidemente in le cellulas del histo extrahepatic e inhibi simultaneemente le transferimento intracellular de glucosa. Tamen, le rapiditate del entrata de 6-FG in le cellulas o su distribution in le aqua de histo non pare esser alterate per le administration de insulina. Similemente, le acceptation de 6-FG per diaphragma de ratto in vitro non es augmentate per le presentia de insulina in le medio de incubation. Es discutite le signification de iste resultatos con respecto al natura del action de insulina.



## ACKNOWLEDGMENT

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## *Restoration of Fat Synthesis after Fasting*

In order to gain further insight into the relationship of fasting and fatty acid synthesis, G. N. Catravas and H. S. Anker (*J. Biol. Chem.* *232*:669, 1958) studied fatty acid synthesis in the livers of fed and fasted female rats. In confirmation of previous results, homogenates from the livers of the fasted rats incorporated only one fourth as much carboxy-labeled acetate as did those from normal rats even under optimal conditions (additional $Mg.^{++}$, nicotinamide, DPN and, especially, citrate). When the homogenates were separated by centrifugation into mitochondria, microsomes and supernatant fluid, it was found that the microsomal fraction was without effect. Mitochondria from both types of animals incorporated only minimal amounts of isotope, and the supernatant fractions only slightly more. Either supernatant fraction plus mitochondria from the fed rats gave maximal incorporation. Since mitochondria from the fasted rats had no effect, it was evidently in this fraction that further search had to be directed.

Boiled extracts of yeast and of liver and liver mitochondria from fed rats were effective in increasing acetate incorporation in homogenates from livers of fasted rats but either did not affect incorporation in those from fed rats or were actually inhibitory. Several likely compounds were tried as activators of fatty acid synthesis, but none proved effective, and yeast extract proved to be the best source of active material. In an attempt to concentrate the activator, the authors passed it, in aqueous solution, through Dowex-1-formate and Dowex-50, effecting a 400-fold purification. Although the actual structure of the active material was not ascertained, it was apparent that it was a polar compound (insoluble in organic solvents) with no charged groups (not absorbed on acidic or basic ion exchange resins) and of fairly low molecular weight (slowly dialyzable).

The authors were able to draw several additional conclusions from these experiments. First, since mitochondria were necessary for maximal fatty acid synthesis and since they alone seemed to carry the activator, it appeared that this fraction is the site of both degradation and synthesis of fatty acids.

Second, it was found, in agreement with the work of others (e.g., I. Zabin, *J. Biol. Chem.* *189*:355, 1951) that although the system from the fed animals apparently effected total synthesis of long chain fatty acids from acetate (since the carboxy carbon had about twice the activity of the average), the homogenates from fasted animals carried out partially an elongation of existing fatty acids (since the carboxy carbon was almost three times the average). Yeast extract restored the normal type of synthetic process.

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# Utilization of Glucose in Normal and Diabetic Rabbits

## Effects of Insulin, Glucagon and Glucose

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In the fasting state the concentration of glucose in blood and extracellular tissues is determined by the balance between the rate of glucose removal by tissue cells and the rate of glucose supply, primarily from liver. The effect of any hypoglycemic agent must therefore be due to an acceleration of glucose removal, a diminution in the rate of supply, or some combination of both. In the intact animal, these two actions are not readily distinguishable if observations are restricted to changes in the blood sugar concentration alone. However, if the blood glucose is labeled with glucose-$C^{14}$, such distinction becomes possible so long as the sources of newly formed glucose remain unlabeled or labeled to an insignificantly small extent.

The chief purpose of the present study has been to assess, with the aid of glucose-$C^{14}$, the relative importance of reduction in glucose supply and increase in glucose utilization in the hypoglycemia induced by insulin. An attempt has been made to evaluate the effects of insulin (a) administered exogenously by subcutaneous and intravenous routes, and (b) secreted endogenously in response to hyperglycemia produced by intravenous glucose or glucagon administration. It has been of further interest to attempt to determine whether insulin is secreted endogenously in response to the administration of certain other sugars and to apply the same methods to the evaluation of the endogenous insulin-secretory response to glucose following a period of insulin therapy.

### TECHNICAL METHODS

Normal untreated rabbits and alloxanized rabbits were used in this study. Intraperitoneal injections of alloxan, 100 mg./kg. body weight, were made daily for four days. Experiments were performed about one week after the completion of alloxan treatment. A fasting blood sugar of over 200 mg. per cent and marked glycosuria for two days prior to the experiment were accepted as criteria of alloxan diabetes. With one exception animals receiving alloxan that did not have both hyperglycemia and glycosuria were omitted from these studies. The fasting blood sugars in the alloxan diabetic rabbits employed were 203, 225, 235, 265, 480, and 480 mg./100 ml. The fasting blood sugar concentrations in untreated animals ranged up to 140 mg./100 ml. but were generally between 80 and 110 mg./100 ml.

All animals were fasted for about eighteen hours prior to study. A single injection of glucose-U-$C^{14}$* was made into an ear vein and at intervals thereafter blood samples were withdrawn from a vein in the opposite ear into syringes moistened with heparin. A crystal of sodium fluoride was added immediately and an aliquot of whole blood was used for determination of reducing sugars by the Nelson modification of Somogyi's method.[1] The remainder of the blood sample was centrifuged and aliquots of plasma were used for measurement of total plasma $C^{14}$ activity, osazone-$C^{14}$ activity and glucose concentration by the glucose oxidase method of Saifer and Gerstenfeld.[2] One-half milliliter of plasma and 2 ml. of distilled water were pipetted into circular aluminum planchets 1¾ in. in diameter, dried under an infrared lamp and assayed for $C^{14}$ in a Nuclear D47 flow gas counter. A measured aliquot of the administered dose of glucose-$C^{14}$, essentially carrier free, was added to duplicate samples and assayed in an identical fashion to provide internal standardization for self-absorption. The sensitivity of this arrangement was approximately $0.25 \times 10^6$ C/M/$\mu$c-$C^{14}$ above a background of 15 C/M.

Osazones were prepared from 1 ml. plasma according to the method of Searle and Chaikoff[3] after the addition of precisely 20 mg. of glucose and were then recrystal-

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From the Radioisotope Service, Veterans Administration Hospital, Bronx, New York, and the Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn, New York.

*Uniformly labeled glucose was purchased from the New England Nuclear Corporation, Boston, Massachusetts.

lized three times. The oven-dried crystals were weighed, dissolved and assayed with and without carrier-free aliquots of the administered dose. The osazone-$C^{14}$ concentration in plasma was calculated from the product of the observed counting rate and the reciprocal of the fraction of total glucose (20 mg. + endogenous glucose per milliliter) represented in the sample counted. Muscle and liver samples were digested in ten to twenty volumes of 10 per cent KOH; one-half or 1 ml. aliquots of the digests were assayed in duplicate, with and without added measured aliquots of the administered dose.

## EXPERIMENTAL OBSERVATIONS

In recent years glucose-$C^{14}$ has been employed widely as a tracer in studies of glucose metabolism in vitro and in vivo. In the intact animal under steady-state conditions, continuous infusion technics are necessary for the precise measurement of glucose utilization, as emphasized by Steele and his colleagues.[4] However, for acute experiments in which the steady state is disturbed, these technics offer no advantage over single injection methods and the latter are more expedient. Nevertheless it must be appreciated that, in acute experiments, either method permits only approximate determinations of glucose pool sizes and turnover rates, owing to the absence of instantaneous equilibration of the tagged glucose throughout the glucose pool.

Following a single intravenous injection of glucose-$C^{14}$ into the rabbit, the plasma concentration of radioactivity falls rapidly at first as the tagged glucose is distributed into the miscible glucose pool. This "distribution" phase is followed within an hour or so by a constant exponential decline (figure 1), the slope of which reflects the rate of glucose removal from the glucose pool and, in the steady state, the rate of glucose turnover. If, now, an experimental procedure results in an increase or decrease in the fractional rate of removal, the slope of the plasma concentration curve becomes sharper or more shallow, respectively.

In initial studies, both total plasma-$C^{14}$ activity and osazone-$C^{14}$ activity were assayed in order to determine the extent to which plasma glucose-$C^{14}$ became contaminated with radioactive products returning to the plasma after metabolic breakdown of the tagged glucose in the tissues. It was observed that, with time, osazone-$C^{14}$ concentrations gradually fell below total plasma-$C^{14}$ concentrations (figure 1), although even after three or more hours the differences were generally less than 10 per cent to 15 per cent; thus turnover rates calculated from each of the two curves were generally in fair agreement. However, as a consequence of these differences, if the fractional rate of glucose removal was accelerated by the administration of insulin or of glucose, the slope of the osazone-$C^{14}$ curve was generally increased to a greater extent than that of the total plasma-$C^{14}$ curve. Under these circumstances glucose removal calculated from the total plasma-$C^{14}$ curve was usually somewhat underestimated compared to calculations from the osazone-$C^{14}$ curve. Osazone preparations are tedious, and in our hands recoveries have been poor, the osazones frequently failing to precipitate after the second or third crystallization. However, since most of the $C^{14}$ activity remaining in the plasma after two or three hours is still glucose-$C^{14}$, we have evaluated glucose removal rates in many instances from the total plasma-$C^{14}$ activity alone, recognizing that the true values are generally somewhat higher.

The concentrations of $C^{14}$ have been plotted as fraction of the dose administered per liter plasma multiplied by the body weight in kilograms so that the reciprocal of the zero time extrapolation (x100) is the apparent glucose space in percentage of body weight.* Glucose spaces ranged from about 20 per cent to 35 per cent of body weight. The "glucose pool" is given by the product of the glucose space and the plasma glucose concentration. Since there were no consistent differences between plasma glucose concentrations determined by the glucose oxidase method and whole blood sugar concentrations determined by the Nelson-Somogyi procedure, the whole blood sugar concentrations were used for the determination of the glucose pool size. The "glucose removal rate" is obtained by multiplying the pool size by the rate constant of the exponential segment of the curve. During the steady-state control period, the blood glucose concentration remains essentially constant, and the rate of removal is equal to the rate of supply. Either is a measure of the turnover rate under fasting control conditions. In normal rabbits most of the values for glucose turnover fell within the range of 80 to 120 mg./kg./hr.† The rate of glucose re-

*It is again emphasized that because of kinetic considerations related to the absence of instantaneous distribution of glucose throughout the glucose pool, measurement of the glucose space by these methods is not precise. However, because of the rapidity of distribution into the major bulk of the glucose space, compared to the rate of turnover of glucose, the error is small. In any event, the same value for glucose space is used for control and experimental periods so that any inherent error does not seriously affect the comparison of results in the two periods.

†In smaller rabbits ($< 3$ kg.) the turnover rate/kg. is somewhat greater.

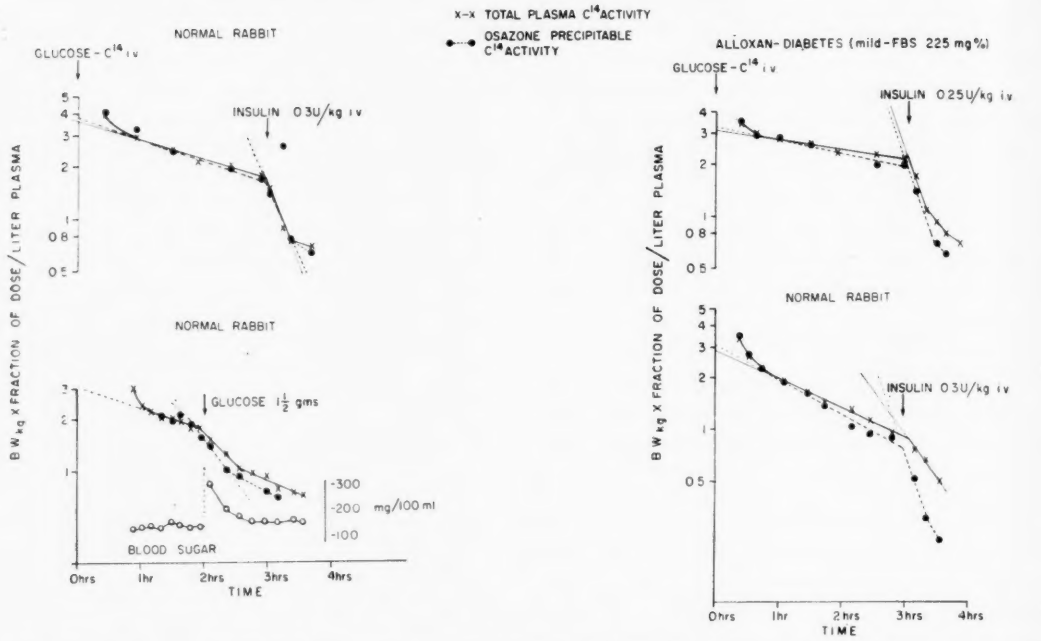


**FIG. 1. Effect of intravenous insulin and intravenous glucose on osazone-$C^{14}$ and total $C^{14}$ concentrations in plasma following glucose-$C^{14}$ administration.**

moval during an experimental period is calculated from the product of the initially determined glucose space, the average glucose concentration during the period and the new rate constant of the plasma-$C^{14}$ curve.

In control studies lasting as long as four to six hours it was observed that the total plasma-$C^{14}$ activity maintained a fairly constant exponential decline (figure 2). If any change was observed, it was a slight tendency toward a decrease in slope. Therefore, increases in slope following an experimental procedure could be attributed only to the effects of such a procedure.

## EFFECTS OF INSULIN

Following the administration of insulin by either the intravenous (figures 1, 2) or the subcutaneous route (figure 3) the slopes of the plasma-$C^{14}$ curves became much steeper, indicative of increases in the fractional rates of glucose removal. Since the blood sugar declines during this period, the increase in glucose removal, calculated as mg./hr., is less marked than the increase in the fractional rate of removal. As noted earlier in dogs[5] and in rabbits,[6] the decline in *labeled* glucose concentration parallels the fall in *unlabeled* sugar concentration, so that the specific activity may remain constant for a short period after insulin administration (figure 2), indicating a cessation of supply of glucose from the liver during this period. However, this was not always evident even when sampling intervals were short, especially after subcutaneous administration of insulin. In some of these cases the slopes of the specific activity curves actually increased (figure 3), indicating negligible effects on sugar release during the period of accelerated removal by the tissues.

## EFFECT OF GLUCOSE

The rapid intravenous administration of 1.5 gm. glucose to normal rabbits produced a sharp increase in slope usually beginning within about twenty minutes (figures 1 and 4). Since the glucose concentration at this time is much higher than during the control period, the glucose removal rate is increased to an even greater extent than is the fractional rate of removal. The increased slope is not related to the elevation in blood glucose concentration per se since the change is usually not evident before fifteen to twenty minutes by which time the glucose concentration has fallen appreciably. It appears most probable that the increase in the fractional rate of removal results from the direct or indirect stimulation of insulin secretion consequent to the hyperglycemia produced, since in alloxan-diabetic animals the

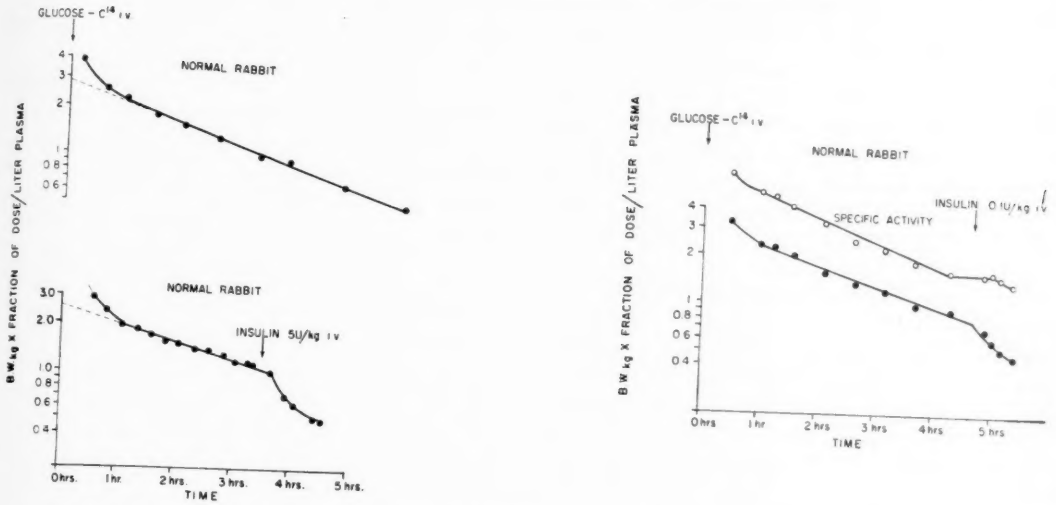


FIG. 2. Plasma-$C^{14}$ concentrations following glucose-$C^{14}$ administration and the effect of intravenous insulin.

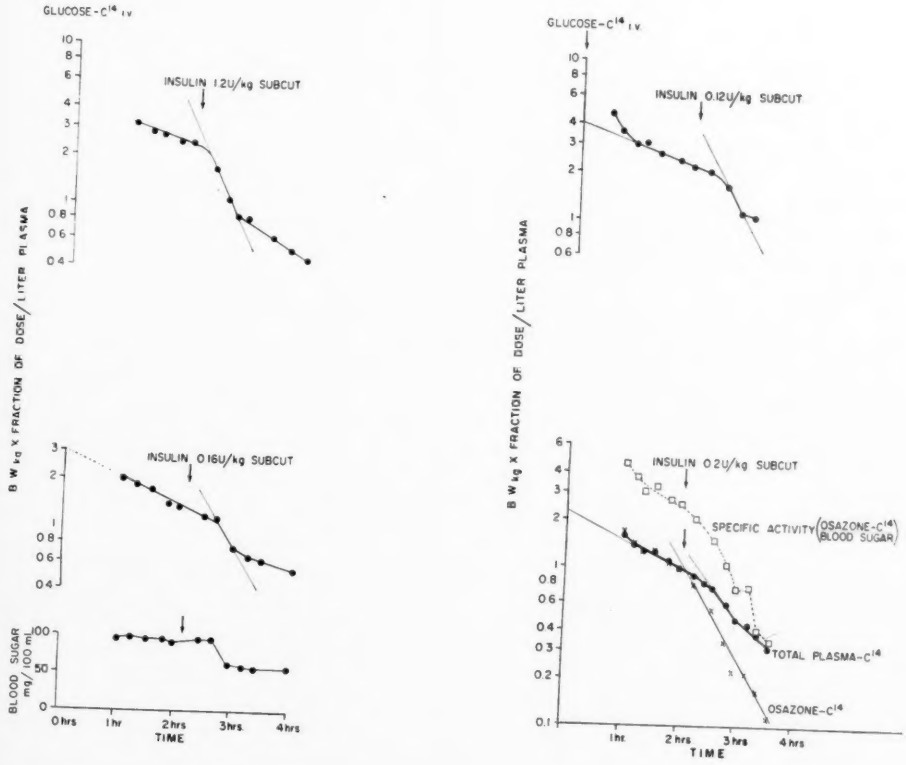


FIG. 3. Effect of subcutaneous insulin on plasma-$C^{14}$ activity following glucose-$C^{14}$ administration.

slope of the curve is actually reduced (figure 4). In the latter cases, total glucose removal changed only slightly if at all. In fact, even in some normal rabbits there is occasionally seen a flattening out of the $C^{14}$ curve immediately following glucose administration and just preceding the sharp increase in slope (figure 4). It appears that the increase in glucose concentration may be too great to permit the tissues to continue to clear glucose at the same fractional rate until insulin is secreted and exerts its effects.

In calculating the rate of glucose removal following administration of glucose, cognizance must be taken of the fact that the glucose concentration in tissues remains lower than that in blood until much of the extra load of glucose has been distributed. The time at which distribution has been essentially completed can be evaluated from an estimate of the peak concentration anticipated after distribution, as calculated from the load administered divided by the glucose space. Calculations of glucose removal rates in these experiments were made only for the period following complete distribution of the administered glucose load. The rate of distribution of the glucose load appeared to be somewhat more rapid than that observed with the original tracer dose, a point of some interest but one which has to be established more definitely by further studies.

Tissue analyses for radioactivity in these animals revealed that the liver contained only about 6 per cent of the total dose administered compared with far greater amounts in muscle (table 1), indicating that hepatic uptake of glucose could not account for the increased rate of disappearance from the plasma.

## EFFECT OF GLUCAGON

Changes in the plasma-$C^{14}$ curve following the intravenous administration of crystalline glucagon (figure 5) were similar to those observed following administration of glucose. Whereas the slope of the curve increased sharply in normal rabbits,* it became more shallow in the alloxan-diabetic rabbit. In the latter case it was observed that after about an hour the slope tended to in-

*The absence of any sharp increase in plasma glucose-$C^{14}$ concentration accompanying the initial rise in blood sugar concentration indicates the absence of any significant labeling of liver glycogen during the interval preceding glucagon administration.

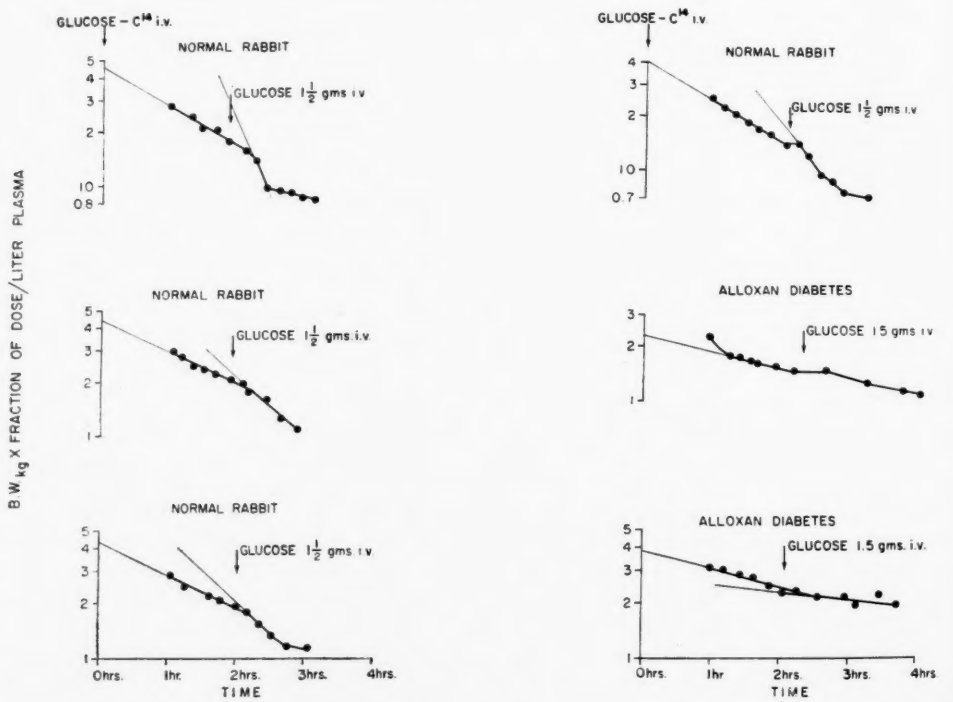


FIG. 4. Effect of intravenous glucose on plasma-$C^{14}$ activity following glucose-$C^{14}$ administration in normal and alloxan-diabetic rabbits.

TABLE 1

$C^{14}$ content of liver and muscle three hours after glucose-$C^{14}$ and one hour after glucose administration

| | Per cent of administered dose $C^{14}$ in whole organ | | |
|---|---|---|---|
| | Liver | Muscle | |
| | | (.35)* | (.45)* |
| Rabbit (a) | 6.30 per cent | 63.5 per cent | 81.5 per cent |
| Rabbit (b) | 6.25 | 36 | 46 |

*Estimated muscle weight as fraction of total body weight. Each value represents the mean result obtained from two specimens.

crease toward that observed during the control period. This was probably due to an increase in renal clearance resulting from the continued rise in blood sugar concentration; whereas glucose utilization alone accounts for turnover in normal animals, glycosuria contributes a significant share to the total glucose removal in diabetic animals.*

It is the failure of an insulin-secretory response to hyperglycemia rather than a toxic effect of alloxan on tissues that is responsible for the absence of an increased fractional rate of removal as demonstrated in a case in which alloxan failed to produce diabetes. Here the response was as marked as in normal nontreated rabbits (figure 5). The flattening out of the slope of the plasma-$C^{14}$ curve just prior to its precipitous increase, mentioned earlier, is also in evidence here (figure 5).

*Only negligible amounts of $C^{14}$ were recovered in the urine of normal rabbits.

## EFFECT OF OTHER SUGARS

The specificity of glucose in stimulating an insulin-secretory response was tested in experiments with other sugars. Galactose, which is itself an insulin responsive sugar,[7] fails to stimulate the secretion of insulin (figure 6). The blood glucose level does not fall nor is there an increase in the slope of the plasma-$C^{14}$ disappearance curve following the intravenous administration of 1.5 gm. of galactose to normal rabbits. In fact, there is occasionally observed a flattening out (figure 6) as might be anticipated if there were competition between glucose and galactose for entry into cells. Fructose likewise failed to stimulate insulin secretion but did not appear to be competitive with glucose under these conditions (figure 7). In two experiments with L-arabinose, another insulin-sensitive sugar, negative results also were obtained for the insulin-secretory response. However, the random spread of the data was too great to warrant calculations of glucose removal rates in these experiments.

## EFFECT OF PRIOR INSULIN THERAPY ON THE RESPONSE TO GLUCOSE

In animals which had been treated with one unit insulin daily for three days, the last dose being given twenty-four hours prior to these studies, there was frequently but not always a failure of response of the plasma-$C^{14}$ curve to glucose administration (figure 8). However, the maintenance of the same slope as observed during the control period in some cases indicated that the extra load was being removed at a rate somewhat greater than during the control period, since

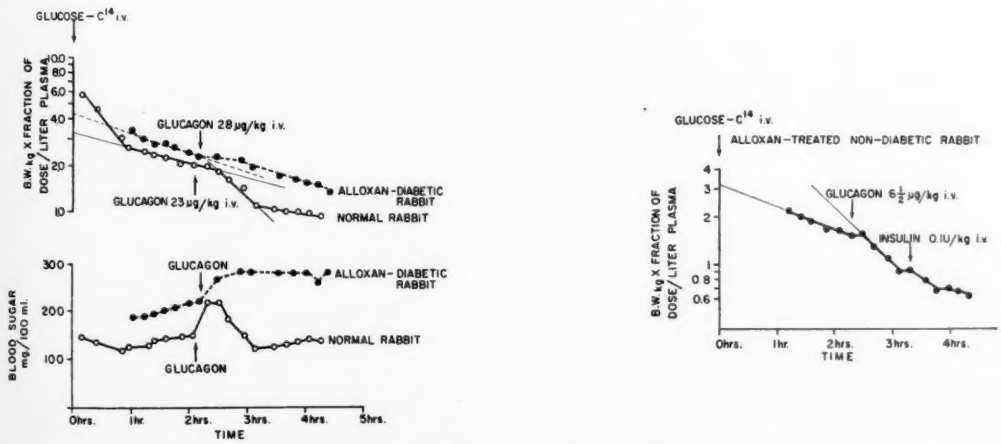


FIG. 5. Effect of intravenous glucagon on plasma-$C^{14}$ activity following glucose-$C^{14}$ administration in diabetic and nondiabetic rabbits.

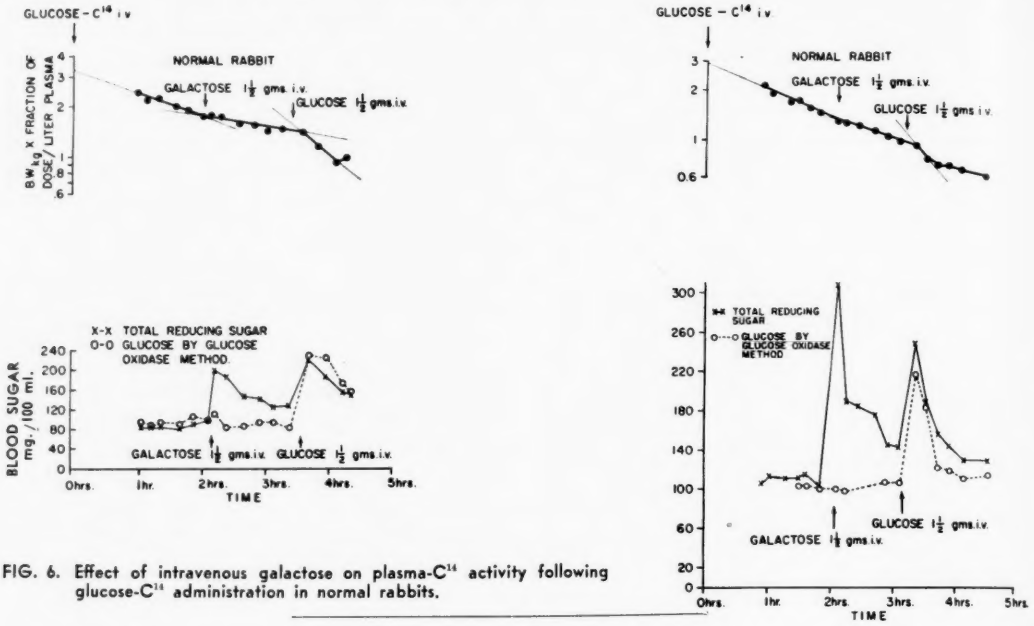
GLUCOSE – C14 i.v.
NORMAL RABBIT
GALACTOSE ½ gms. i.v.
GLUCOSE ½ gms. i.v.
B.W.kg X FRACTION OF DOSE/LITER PLASMA
X-X TOTAL REDUCING SUGAR
O-O GLUCOSE BY GLUCOSE OXIDASE METHOD
BLOOD SUGAR mg./100 ml.
TIME
0hrs. 1hr. 2hrs. 3hrs. 4hrs. 5hrs.

FIG. 6. Effect of intravenous galactose on plasma-$C^{14}$ activity following glucose-$C^{14}$ administration in normal rabbits.

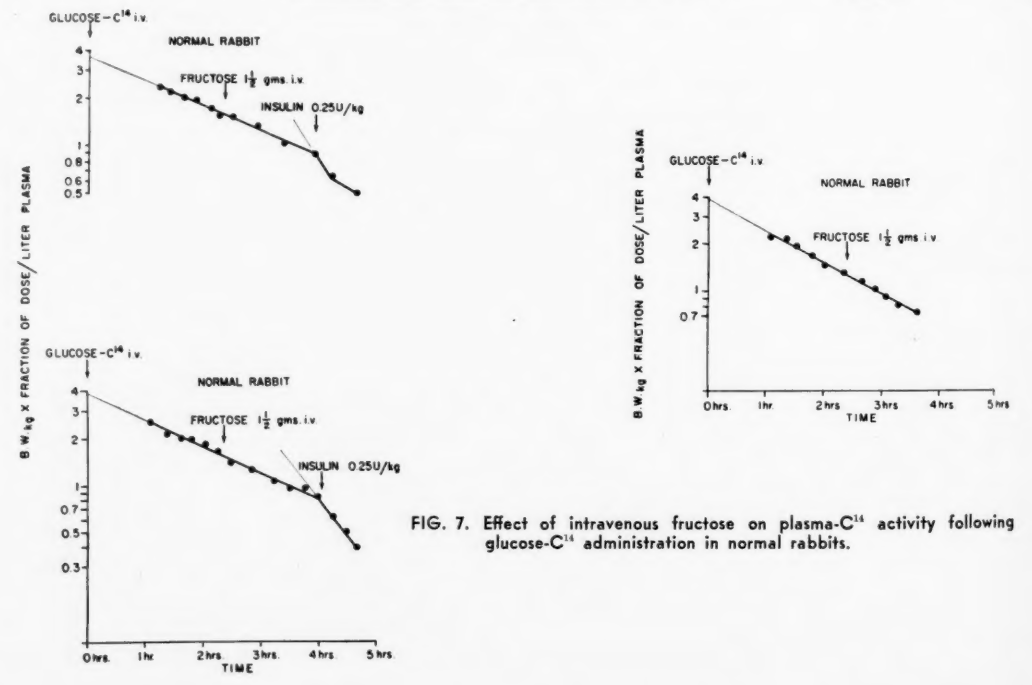


FIG. 7. Effect of intravenous fructose on plasma-$C^{14}$ activity following glucose-$C^{14}$ administration in normal rabbits.

the average higher blood sugar levels followed glucose administration.

The data on glucose removal during control and experimental periods are summarized in figures 9-11. It is clear that following administration of insulin (figure 9) or of glucose (figure 10) to normal rabbits, the rate of removal generally exceeds that observed during the fasting control period by more than 100 per cent at

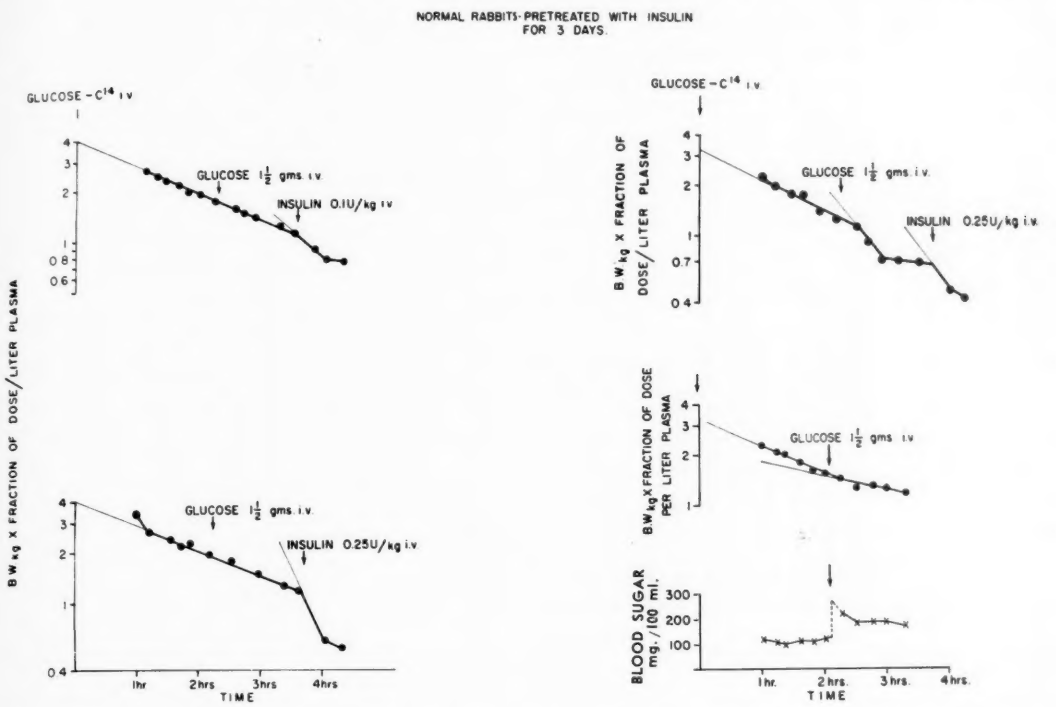


FIG. 8. Effect of intravenous glucose on plasma-$C^{14}$ activity following glucose-$C^{14}$ administration in normal rabbits pretreated with insulin for three days.

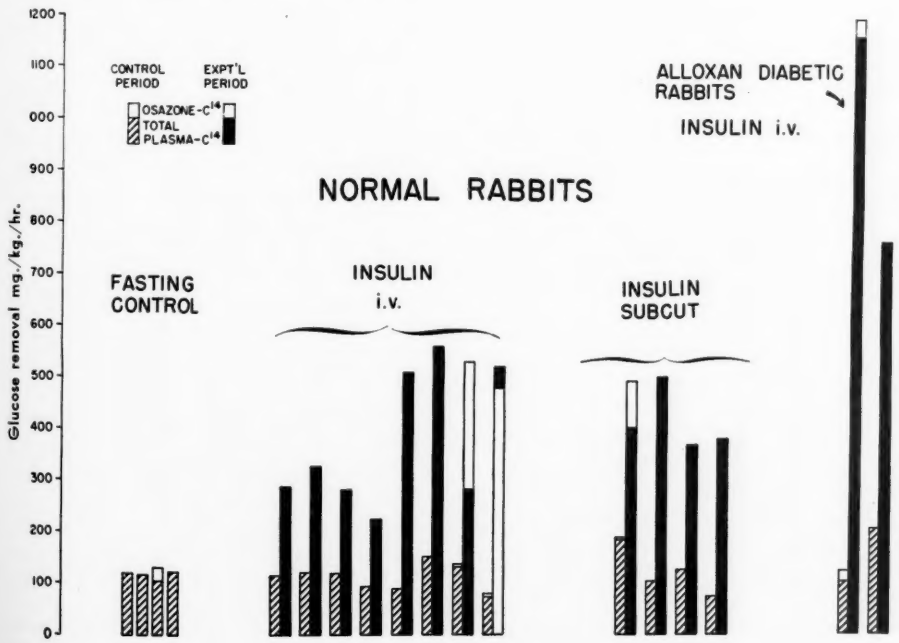


FIG. 9. Effect of intravenous and subcutaneous insulin on glucose removal from the glucose "pool" in normal and diabetic rabbits. The values in diabetic rabbits include unmeasured urinary losses.

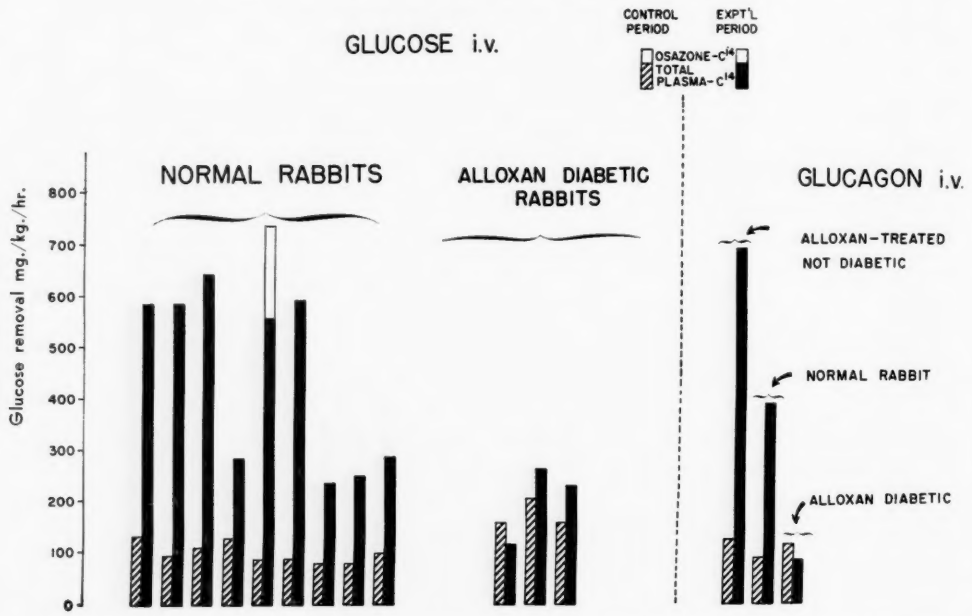


FIG. 10. Effect of intravenous glucose and glucagon on glucose removal from the glucose "pool" in normal and diabetic rabbits. The values in diabetic animals include unmeasured urinary losses.

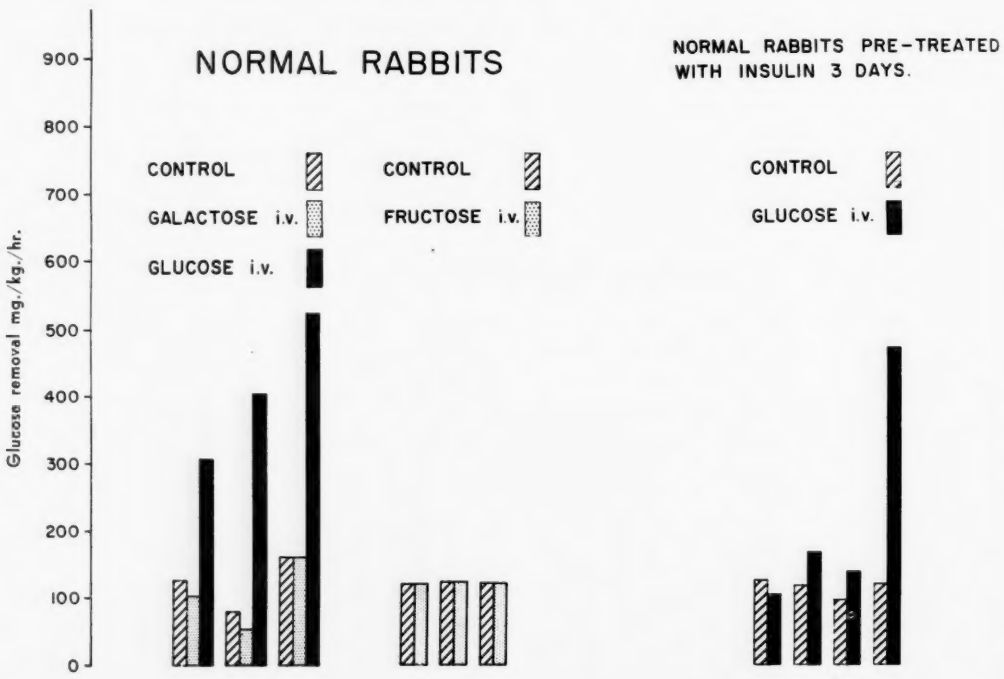


FIG. 11. Effect of intravenous galactose and fructose on glucose removal from the glucose "pool" in normal rabbits and the effect of intravenous glucose in normal rabbits pretreated with 1 unit insulin daily for three days.

least and occasionally by as much as 500 to 600 per cent. Even if glucose release from the liver were completely inhibited, as is occasionally observed for a short period, increased removal must still be held primarily responsible both for the restoration of normal blood sugar concentrations following glucose administration and for the decline in blood sugar following insulin administration.

## DISCUSSION

In depancreatized dogs given large doses of insulin (80 units intravenously), Henderson, Wrenshall and Odense[5] reported a marked immediate increase in glucose utilization as measured with glucose-$C^{14}$, but only a small immediate decrease in the hepatic release of glucose (17 per cent at fifteen minutes) which became progressively more noticeable with time. The specific activity curves did not show an abrupt flattening out. However, Dunn, Friedmann, Maass, Reichard and Weinhouse[5] observed that the specific activity curves flattened out and maintained a plateau for ten to twenty minutes immediately following intravenous administration of 3 to 10 units of insulin to normal dogs, and on this basis they concluded that there was a complete cessation of glucose supply to the blood during this period. From observations showing an almost identical rate of decline in plasma-$C^{14}$ activity and blood glucose concentration immediately following intravenous administration of insulin to normal rabbits, Berson and Yalow[6] also concluded that insulin may effectively inhibit glucose release from the liver.

In all the studies just cited, the increase in glucose utilization played a more significant role in the hypoglycemia induced by insulin than did inhibition of glucose production. However, more recently Jacobs, Reichard, Goodman, Friedmann and Weinhouse[9,10] have reported that when insulin is administered subcutaneously to humans there is no increase in glucose utilization and concluded that the hypoglycemia is to be attributed solely to diminution in the rate of glucose release from the liver. The findings of the present study are in disagreement. Whether insulin was administered intravenously or subcutaneously, a marked increase in the rate of glucose utilization was regularly observed and generally exceeded by far the diminution in hepatic glucose supply. Although an apparent complete cessation of glucose supply was frequently observed following insulin administration, the effect was usually short-lived and occasionally absent.

Although the different species employed in the studies of Weinhouse et al. and those reported here may account for the different results, it appears more likely that differences in the methods of calculation might be responsible. In the earlier reports of Weinhouse and his colleagues[5,11] the control glucose turnover rate was calculated from the slope of the specific activity curve during the first forty-five to sixty minutes following injection of glucose-$C^{14}$. As is evident from the results of the present study, such a procedure in the rabbit would lead to a significant overestimation of the control glucose turnover rate. Since glucose-$C^{14}$ distribution is rarely complete in less than thirty to sixty minutes, the fall in specific activity during the first hour is largely due to mixing of glucose-$C^{14}$ with extravascular glucose rather than to glucose utilization alone.* In one experiment of Reichard and his associates[11] in which the control period lasted eighty minutes, the published figure (figure 4 in reference 11) reveals a continually decreasing slope which ultimately became much more shallow than the slope employed for the calculation of glucose utilization. It is evident that increased utilization induced by experimental procedures might well go undetected if utilization during the control period is overestimated.

Reichard et al.[11] have also interpreted the temporary flat portion of the specific activity curve following glucose administration as indicating a cessation of glucose production. While it is not our purpose to dispute this conclusion, it may be questioned whether such observations provide unequivocal evidence of diminished hepatic release of glucose under these conditions. Following the intravenous injection of unlabeled glucose, the specific activity in the blood must fall abruptly simply as a result of the markedly increased concentration of unlabeled glucose. As the newly administered unlabeled glucose distributes into extravascular spaces, the specific activity in the blood must again rise as the blood glucose concentration falls. (Since the previously administered *labeled* glucose has already achieved distribution equilibrium, the glucose-$C^{14}$ concentration remains essentially constant.) As distribution of the newly administered unlabeled glucose nears completion the rate of increase in specific activity of the blood diminishes and can be just offset by a decrease in specific activity that results from continued glucose supply to the blood from the liver. Thus a short period in which the specific activity in the blood remains constant following unlabeled glucose injection is an obligatory consequence of the kinetics of glucose mixing and distribu-

*It is also to be noted that in the studies of Shreeve et al.[12] in human subjects, the phase of glucose-$C^{14}$ mixing did not appear to be complete for at least an hour.

tion even if there is no inhibition of glucose production. For this reason we have been unwilling to make any calculations of hepatic glucose production from changes in specific activity following glucose administration. However, the rates of glucose utilization in normal animals were increased so greatly by glucose administration as to exceed the diminution in glucose production even if the latter were completely abolished.

It was suggested by Jacobs et al.[9,10] that subcutaneous administration more nearly simulates the secretion of endogenous insulin from the pancreas in providing a slow continued supply to the blood stream, and therefore, that the "primary action of insulin in the physiologic regulation of blood glucose may be to control hepatic glucose output" rather than to augment peripheral glucose utilization. However, the experiments of the present study reveal that glucose utilization is increased several fold following the administration of glucose or glucagon to normal rabbits and marked increases in utilization under similar conditions were observed also by Reichard et al.[11] in dogs. Such findings imply that endogenous insulin secretion in response to hyperglycemia does in fact augment glucose utilization.

It is not necessary to recount here the evidence that hyperglycemia is responsible for stimulation of insulin secretion in normal animals. Foa[12] has recently contributed a critical review on this subject and has summarized evidence that the mechanism of action may be explained by a direct effect of hyperglycemia on the pancreas. In the present study, the increase in the *fractional* rate of disappearance of glucose-$C^{14}$ in normal animals and the decrease in alloxan-diabetic animals given glucose or glucagon indicate that the increased glucose utilization rate is not the result of hyperglycemia per se but must be attributed to insulin secretion. The absence of an increase in the fractional rate of removal of glucose-$C^{14}$ following the administration of fructose, D-galactose or L-arabinose to normal animals indicates that these sugars fail to stimulate insulin secretion under the conditions of these experiments. The poor insulin-secretory response to glucose (as manifested by suboptimal increase in glucose utilization and the frequent absence of an increase in the fractional rate of glucose-$C^{14}$ removal) in rabbits treated with insulin for several days is consistent with the diminished insulin content in the pancreas of animals treated with insulin.[14]

## SUMMARY AND CONCLUSIONS

1. Following a single intravenous injection of glucose-$C^{14}$ in rabbits the shape of the plasma concentration curve indicates an initial phase of mixing with extravascular glucose, which is not complete for at least thirty to sixty minutes, followed by an exponential decline in concentration attributable to glucose turnover.

2. Estimates based on the apparent volume of distribution of glucose-$C^{14}$ and the fractional rate of turnover of the miscible glucose pool yield values for glucose turnover averaging about 80-120 mg./kg./hr. in normal rabbits weighing about 4 kg.

3. The subcutaneous or intravenous administration of insulin to normal or diabetic rabbits results in a marked increase in the slope of the glucose-$C^{14}$ turnover curve and accordingly to an increase in the calculated values for glucose utilization. Frequently, but not always, there is an abrupt decrease in the slope of the specific activity curve following insulin injections indicating a diminution or cessation in glucose production.

The intravenous injection of glucose or of effective doses of glucagon results in a marked increase in the rate of glucose utilization in normal animals but not in alloxan-diabetic animals. The changes in plasma concentration of glucose-$C^{14}$ are interpreted as resulting from the stimulation of endogenous insulin secretion in normal animals and the absence of such in diabetic animals.

5. Intravenous injection of D-galactose, fructose or L-arabinose was ineffective in stimulating endogenous secretion of insulin as determined by changes in plasma glucose-$C^{14}$ concentration. A diminished rate of glucose-$C^{14}$ removal from plasma following galactose injection suggested that galactose might be competitive with glucose for entry into cells.

6. Suboptimal increases in the rate of removal of plasma glucose-$C^{14}$ in response to glucose injections were observed in normal rabbits treated with small doses of insulin for three days.

## SUMMARIO IN INTERLINGUA

*Effectos De Insulina, Glucagon, E Glucosa Super Le Utilisation De Glucosa In Normal E In Diabetic Conilios*

1. Post un unic injection intravenose de glucosa a $C^{14}$ in conilios, le conformation del curva de su concentration in le plasma indica un phase initial de mixtion de glucosa endogene con glucosa extravascular. Iste phase es non jam complete post trenta a sexanta minutas. Post su termination il occurre un reduction exponential del concentration de glucosa, le qual es attribuibile al metabolismo de glucosa.

2. Estimationes basate super le apparente volumine de distribution de glucosa a $C^{14}$ e le mesura fractional del metabolismo del miscibile reservoir de glucosa resulta in valores pro le metabolismo de glucosa amontante, al media, a circa 80 a 120 mg per kg per hora in

conilios normal de un peso de circa 4 kg.

3. Le administration subcutanee o intravenose de insulina a normal o diabetic conilios resulta in un marcate augmento del declino del curva de metabolismo de glucosa a $C^{14}$ e assi in un augmento del calculate valores pro le utilisation de glucosa. In multe sed non in omne casos, il occurre un reduction abrupte in le declino del curva de activitate specific post injectiones de insulina. Isto indica un diminution o cessation del production de glucosa.

4. Le injection intravenose de glucosa o de doses efficace de glucagon resulta in un augmento marcate in le mesura del utilisation de glucosa in animales normal. Isto non occurre in animales rendite diabetic per medio de alloxano. Le alterationes in le concentration de glucosa a $C^{14}$ in le plasma es interpretate como resultato de un stimulation del secretion de insulina endogene in animales normal e del absentia de iste phenomeno in animales diabetic.

5. Le injection intravenose de D-galactosa, de fructosa, o de L-arabinosa esseva inefficace in le stimulation del secretion endogene de insulina, secundo determinationes del concentration de glucosa a $C^{14}$ in le plasma. Un relentate elimination de glucosa a $C^{14}$ ab le plasma post injectiones de galactosa suggereva le possibilitate que galactosa es un competitor de glucosa quanto al entrata a in le cellulas.

6. Grados suboptimal de intensification del elimination de glucosa a $C^{14}$ ab le plasma in responsa a injectiones de glucosa esseva observate in conilios normal que habeva essite tractate durante tres dies con micre doses de insulina.

ACKNOWLEDGMENT

We are indebted to Miss Ida Fuhrer for technical assistance, to Mrs. Frieda Steiner for secretarial assistance, and to Mrs. Ellen Pudvan, Miss Bess Bonner, Mr. David Lubin, Mr. William Greenberg and Mr. Ray Lookofsky of the Medical Illustration Department of the Veterans Administration Hospital, Bronx, New York, for the figures.

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Incidentally, biochemistry makes strange bedfellows of genetic diseases. Hereditary spherocytosis is now seen to be intimately related biochemically to galactosemia and the glycogen diseases through glycolytic metabolism, just as albinism, phenylketonuria, tyrosinosis, and alcaptonuria are all, surprisingly, biochemically related to one another through the metabolism of phenylalanine, one of the amino acids. In spite of the close biochemical relationships, however, the inheritance of such related disorders is quite independent and specific.

Laurence H. Snyder, in "Fifty Years of Medical Genetics," from *Science*, Jan. 2, 1959.

# Glucagon Content of Glucagon-Insulin Mixtures

## Estimation Using Cortisone-pretreated Animals

*Bruno W. Volk, M.D., and Sydney S. Lazarus, M.D., M.Sc. (Med.), New York City*

In previous work, the authors[1,2] showed that the administration of large doses of regular insulin to rabbits pretreated with cortisone or pituitary growth hormone caused a marked protracted hyperglycemia followed by the expected decline in the blood sugar level. This initial increased glycemia which greatly exceeded that occurring in untreated rabbits was shown to be due to a potentiation by cortisone of the hyperglycemic action of the small amounts of glucagon contained in commercial insulin. It was further shown that with mixtures of glucagon and insulin, the maximal hyperglycemia depended on the glucagon content of the mixture and was independent of its insulin content. These data suggested that the cortisone pretreated rabbit could be utilized for assay of the glucagon content of such glucagon-insulin mixtures. Other workers[3] have reported that glucagon added to insulin does not affect the assay of insulin by either the mouse or rabbit method. However, our experiments indicated that this might not be true for cortisone pretreated animals because of their markedly greater response to glucagon. A study was therefore undertaken to determine whether either the mouse or rabbit insulin assay[4] might be adaptable through cortisone pretreatment of the animals for the assay of glucagon when present in insulin.

### EXPERIMENTAL

I. Studies in Mice

(A) *Untreated*: Two sets of twenty untreated white mice weighing from 14 to 22 gm. were fasted from 9 a.m. to 3 p.m. Each group of twenty was divided in half and the effect of two different test dosages (T1 and T2) of Glucagon-Free Insulin (see footnote †, column 2) (GFI) and of Regular Commercial Insulin (RI) (Lilly) was compared. Immediately after subcutaneous injection of the test material the mice were placed in an incubator at 37° C. The animals were observed for two hours and the number of each set which had convulsed or died was noted. The test material consisted of either .06 or .08 units of RI or similar doses of GFI. All animals in each set convulsed (table 1).

(B) *Treatment with a single dose of cortisone acetate*: Sets of mice which were otherwise treated as in (A) received in addition 0.5 mg. of cortisone acetate in aqueous suspension* at 9 a.m., subcutaneously, on the morning of the experiments. The test materials (T1 and T2) are indicated in table 1, as well as the percentage of each group convulsing. Analysis of these data showed no difference between the effect of GFI alone and of GFI with added amorphous glucagon.†

(C) *Pretreatment with cortisone acetate for seven days*: Ten additional sets of mice were pretreated with cortisone acetate 0.5 mg. daily for seven days. On the seventh day the animals were tested as in (A). The insulin and glucagon dosages for each set (T1 and T2) are indicated in table 1 as well as the percentage which convulsed. Analysis of these data showed no significant differences due to the added glucagon. Similarly, when the time between the injection of the test material and the onset of the convulsions was compared, no valid differences were obtained.

II. Studies in rabbits

(A) *Untreated*: Rabbits of either sex weighing between 2.5 and 3.5 kg. were divided into sets of six. Each set was further divided into two subgroups of three each. On the first experimental day, one of these subgroups received intravenously test dose 1 (T1), the other was given test dose 2 (T2). After a one-day rest period the animals were again fasted eighteen hours and the tests were repeated except that the second subgroup now received T1 and the first subgroup was given T2. In each instance two blood samples for glucose determination by the Nelson-Somogyi micromethod[5] were taken at fifteen-minute intervals prior to administration of the test material and thereafter at hourly intervals for five hours. The different sets of untreated rabbits and their respective test doses are indicated in table 2, columns 1, 2 and 3.

From the Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn, New York, and the Department of Pathology, Albert Einstein College of Medicine, Bronx, New York.

* The cortisone acetate was supplied by The Upjohn Company, Kalamazoo, Michigan, through the courtesy of Dr. C. J. O'Donovan.

† The glucagon-free insulin as well as amorphous glucagon (Lot No. 208-158B-197, potency 50 per cent by weight as compared with crystalline glucagon) was supplied by the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, through the courtesy of Dr. Otto K. Behrens.

TABLE 1
Summary of mice experiments

| Experiment | Total number of mice | Test dosage (T) T1 (GFI*) | Test dosage (T) T2 | Results: Per cent convulsed or died T1 per cent | Results: Per cent convulsed or died T2 per cent |
|---|---|---|---|---|---|
| (A) No pretreatment | 20 | 0.06 | 0.06 RI† | 100 | 100 |
| | 20 | 0.08 | 0.08 RI | 100 | 100 |
| (B) Pretreatment with single dose of 0.5 mg. of cortisone acetate | 20 | 0.06 | 0.06 RI | 30 | 30 |
| | 40 | 0.08 | 0.08 RI | 50 | 47.4 |
| | 40 | 5.0 | 5.0 RI | 85.7 | 60.0 |
| | 120 | 0.25 | 0.25 GFI + 0.5 to 15 G‡ | 62.7 | 61.4 |
| | 40 | 0.5 | 0.5 GFI + 15 G | 80 | 70 |
| | 80 | 1.0 | 0.5 GFI + 2 to 15 G | 87.5 | 90 |
| (C) Pretreatment with 0.5 mg. daily of cortisone acetate s.c. for seven days prior to test day | 120 | 0.5 | 0.5 GFI + 30, 60 or 100 G | 71 | 60 |
| | 80 | 1.0 | 1.0 GFI +100 or 500 G | 75.9 | 65.5 |

*GFI = Dose of glucagon-free insulin in units.
†RI = Dose of regular commercial insulin in units.
‡G = Dose of amorphous glucagon in micrograms. This material has 50 per cent of the potency of crystalline glucagon.

(B) *Pretreatment with cortisone acetate*: Rabbits which received 5 mg./kg. of cortisone acetate intramuscularly for seven days prior to experimental day 1 were divided into sets of six and tested as above (table 2).

For analysis of the blood sugar changes in the rabbits, the average of the two fasting blood sugar values was subtracted from the average of the five postinjection values and the percentage change in blood sugar concentration determined. The mean percentage change in blood sugar for each test material (T1 and T2) in each set of six rabbits was then calculated. An analysis of variance for each set was performed giving the average standard error of means T1 and T2 as well as the standard error for the difference in blood sugar response (T2-T1). The variance ratio (F) was utilized to determine the significance of this difference.[6] The results are indicated in table 2 together with the F values, as well as their significance which was determined from the tables of F values.[7] It can be seen that glucagon added to insulin did not significantly reduce its hypoglycemic effectiveness in untreated rabbits. On the other hand, in cortisone-treated rabbits all three glucagon dosages significantly altered the mean response to insulin. Ten micrograms of amorphous glucagon added to 20 units of GFI reduced the mean hypoglycemia to —19.4 per cent of the fasting value as compared with —42.8 per cent of fasting with GFI alone. This difference of —23.4 per cent is significant at the 5 per cent level. One hundred and 500 μg. of added amorphous glucagon not only inhibited hypoglycemia but instead produced a mean hyperglycemia of +17 per cent and +30 per cent of the fasting value. In these later instances the response differences of +57.3 per cent and +53.5 per cent are significant at the 1 per cent level. When the alteration of hypoglycemia induced by adding 10 μg. of amorphous glucagon to 20 units of GFI was compared with that due to addition of 100 μg. of glucagon the difference was not significant. On the other hand, the difference between the effect of addition of 100 and 500 μg. of amorphous glucagon was significant (table 2).

## DISCUSSION

The present study is an extension of previous work with the aim of eventually developing a biologic assay for glucagon which is less arduous and more easily reproducible than those used at present.[8-10] The liver slice method as originally described[8] is only semiquantitative. The slice homogenate method[10] utilizing a five-point assay gives confidence limits for $P<0.05$ of $\pm 7.4$ per cent. In this method the insulin present in the extract must be inactivated, thus also subjecting the glucagon to harsh chemical treatment which tends to reduce its activity. The cat assay[9] also suffers from the latter objection and in addition due to the marked variability in extent of blood sugar response to the same glucagon dose in different animals showed confidence limits for $P<0.05$ of $\pm 11$ per cent to $\pm 24$ per cent in assays using as many as fourteen to nineteen cats.

The present finding that the untreated mouse or rabbit responds similarly to insulin alone and to insulin admixed with glucagon is in accord with reports[8] that glucagon does not affect the assay of insulin by the mouse or rabbit method. Moreover, support was ob-

TABLE 2
Summary of rabbit experiments

| | Test material | | Mean percentage change in blood sugar[1] | | | Percentage response difference | | Variance ratio | |
|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T1 | T2 | S.E. of mean (T1 and T2)[2] | y = T2-T1 | $S.E_y$[3] | $F_y$[4] | Significant |
| (A) No pretreatment | 20 GFI* | 20 GFI + 10 G | —59.6 | —66.9 | ± 4.8 | — 7.3 | ± 6.7 | 1.24 | NS |
| | 20 GFI | 20 GFI +100 G | —57.4 | —40.4 | ± 6.4 | +17.0 | ± 9.1 | 1.60 | NS |
| | 20 GFI | 20 GFI +500 G | —68.8 | —53.4 | ± 6.0 | +15.4 | ± 8.5 | 3.31 | NS |
| (B) Pretreated with cortisone acetate 5 mg./kg. daily, i.m. for seven days | 20 GFI | 20 GFI + 10 G | —42.8 | —19.4 | ± 7.2 | +23.4 | ±10.1 | 5.48 | <.05 |
| | 20 GFI | 20 GFI +100 G | —29.0 | +30.3 | ± 9.8 | +59.3 | ±13.8 | 18.60 | <.01 |
| | 20 GFI | 20 GFI +500 G | —36.5 | +13.6 | ± 9.8 | +50.1 | ±13.8 | 13.05 | <.01 |
| | 20 GFI +10 G† | 20 GFI +100 G | + 3.5 | +27.5 | ±13.1 | +23.8 | ±18.5 | 1.7 | NS |
| | 20 GFI +100 G | 20 GFI +500 G | + 9.7 | +64.8 | ±11.5 | +55.1 | ±16.3 | 11.4 | <.01 |

*GFI = Glucagon-free insulin in units.
†G = Amorphous glucagon in micrograms which has 50 per cent potency as compared with crystalline glucagon.

[1] Mean of percentage change in blood sugar in the six rabbits of each group after test material 1 (T1) and after test material 2 (T2).

$$\text{Per cent change in blood sugar} = \frac{100 \times \text{I.B.S. (Initial Blood Sugar)} - \text{F.B.S. (Final Blood Sugar)}}{\text{I.B.S.}}$$

I.B.S. = Average of two preinjection blood sugar values taken at fifteen-minute intervals.
F.B.S. = Average of five postinjection blood sugar values taken at hourly intervals.

[2] Average standard error of mean = $\sqrt{\frac{\text{within groups variance}}{N}}$

[3] Standard error of difference ($S.E._y$) = $\sqrt{\frac{2 \times \text{within groups variance}}{N}}$

[4] P = .05, F (1,8) = 5.32
P = .01, F (1,8) = 11.26

tained for previous observations that cortisone pretreatment of rabbits potentiates the hyperglycemic effect of glucagon. Furthermore, it is demonstrated that with adequate amounts of glucagon marked prolonged hyperglycemia may be observed even in the presence of a large dose of insulin. Previous work[1,2] demonstrated that the extent of hyperglycemia became maximal with glucagon dosages of approximately 50 μg. Increasing the dosage prolonged the duration but did not influence the extent of the hyperglycemia. The timing of blood sugar samples in the rabbit experiments allows both the extent and duration of hyperglycemia to be taken into account for determining glucagon activity.

The dosage of GFI for the rabbits was arbitrarily fixed at 20 units because this dose consistently produced a marked diminution of fasting blood sugar in the cortisone-treated rabbit with comparatively small mortality. This amount of insulin was not in itself large enough to cause hyperglycemia due to its glucagon content. Furthermore,[1,2] an increase in insulin dosage might have paradoxically diminished rather than enhanced the extent of hypoglycemia. Also, we have previously shown that the blood sugar response to insulin is proportional to the dose only up to a certain dosage level.[11]

The main assumption in any assay is that the experimental conditions are such that observed differences are an effect of the material being tested. However, animals vary in their responses both among themselves and from day to day. The use of the crossover design in which each rabbit is tested with each of two preparations tends to eliminate variations due to differences in sensitivity of individual animals. However, variations due to testing on different days may appear. Although usually not significant, there were in the present study occasional instances in which a significant difference was obtained due to differences in days of testing. It is believed that this occurred because glucagon, even in the cortisone-treated animal, has maximal effectiveness only when the animal is well fed. Since the animals were fasted on alternate days with only one day of feeding in

between, it is important to start refeeding the rabbits immediately after completion of the experiment carried out on the first day.

The failure to detect a difference between the cortisone-treated and the untreated mice is assumed to be due to the use of convulsions as the end point and to the fact[1,2] that the eventual hypoglycemia is the same with insulin alone as with insulin together with glucagon. It is only the time of appearance of the hypoglycemia that is delayed. Furthermore, convulsions do not necessarily reflect the extent of hypoglycemia. It has previously been shown by us[12] that under certain conditions in humans, hypoglycemic reactions do not occur with blood sugar levels of 18 to 25 mg. per 100 ml. In addition, it has been stated by others that the onset of hypoglycemic reaction may be determined by the concentration of glucose in the tissues rather than its concentration in the blood.[13]

The results obtained with the rabbits indicate that this method may be developed into an assay for glucagon even when present together with large amounts of insulin. This probably could be accomplished by finding the minimal amount of unknown material which when injected with a standard dose of GFI (20 units) would cause a significant reduction in the resultant hypoglycemia. From the present data it is probable that approximately 5 μg. of crystalline glucagon could be detected.

## SUMMARY

A study is reported to determine whether, using cortisone pretreated mice or rabbits, an assay for glucagon might be developed. In either normal or cortisone pretreated mice or in normal rabbits glucagon caused no significant difference in the response to administration of insulin. But, distinct differences were obtained with cortisone pretreated rabbits. This enabled the differentiation of insulin containing a minimum of 10 μg. of amorphous glucagon (50 per cent potency as compared with crystalline glucagon) from glucagon-free insulin and also of insulin with 100 μg. of amorphous glucagon from insulin containing 500 μg. It is believed that the cortisone pretreated rabbit could be utilized for the assay of glucagon even when present in insulin preparations.

## SUMMARIO IN INTERLINGUA

*Estimation Del Contento De Glucagon In Mixturas De Glucagon E Insulina, Con Le Uso De Animales Pre-Tractate Con Cortisona*

Es reportate un studio concernite a determinar si, con le uso de muses o conilios pre-tractate con cortisona, un methodo pro le essayage de glucagon pote esser disveloppate. In muses e conilios normal e etiam in muses pre-tractate con cortisona, glucagon causava nulle significative differentia in le responsa al administration de insulina. Del altere latere, distincte differentias esseva constatate in le caso de conilios pre-tractate con cortisona. Isto permitteva le differentiation de insulina a contentos de glucagon amorphe usque al minimo de dece microgrammas ab insulina totalmente libere de glucagon. (Glucagon amorphe ha un potentia de 50 pro cento de glucagon crystallin.) Similemente il esseva possibile differentiar insulina continente 100 microgrammas de glucagon amorphe ab insulina continente 500 microgrammas. Es formulate le opinion que conilios pre-tractate con cortisona pote esser utilisate pro le essayage de glucagon mesmo quando le glucagon es continite in preparatos de insulina.

## ACKNOWLEDGMENT

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# Ketogenic Action of Pituitary and Adrenal Hormones in Pancreatectomized Rats

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Severe diabetes with marked alterations of fat metabolism develops in several species following total extirpation of the pancreas.[1,2] The classical experiments of Houssay and of Long and Lukens clearly demonstrated that the anterior pituitary and adrenal glands are necessary for the development of diabetes in such animals.[1,2] There have been few studies, however, to determine which hormones are involved in this process, especially in the development of ketosis and accumulation of fat in the blood, liver and kidneys.[3,4] It was recently demonstrated that severe diabetes culminating in coma and death develops in well-nourished rats when they are deprived of 99.5 per cent of the pancreas.[5] This preparation has been used in the present experiments to study the ketogenic and adipokinetic action of the pituitary and adrenal hormones in diabetes.

## METHODS

Female Sprague-Dawley rats, weighing 150-170 gm., were fed ad libitum for one to three weeks a diet (No. 4370) consisting of casein 20 per cent, dextrin 67 per cent, fat 8 per cent, and all salts and vitamins needed by the rat.[5] After a seventeen-hour fast 99.5 per cent of the pancreas was removed under ether anesthesia by the procedure of Scow.[5] The previously described postoperative care was modified so that by the fourth day each rat received twice daily 12 units of regular insulin subcutaneously and 4.5 gm. of food (diet 4370) and 90 mg. of pancreatin by stomach tube (figure 1). There was an interval of about six hours between meals.

The pancreatectomized rats were on this regimen for seven to twelve days prior to the experimental period; one group, P-80, was maintained for eighty days (figure 2). Rats with jaundice or with blood phospholipid levels of more than 400 mg. per cent at the beginning of the experimental period were not included in the data presented in this paper. The pronounced effect of biliary obstruction on the blood triglycerides in the insulin-deficient rat has been described in a recent publication.[7]

The experimental period was begun seventeen hours after the last insulin injection and meal, and lasted up to two days. At that time either the pituitary or adrenal glands were removed and one to two hours later the first hormone injections were given. The second injection of all hormones except ACTH was given about twenty hours later; ACTH was given three to four times per day. Ten milliliters of 0.9 per cent NaCl solution were given subcutaneously twice on the first day and once on the second day. The adrenalectomized rats were given 0.9 per cent NaCl solution to drink ad libitum; the others, water.

The growth hormone* was dissolved in water at *p*H 9.4. The other pituitary hormones, adrenocorticotrophic (ACTH),† thyroid stimulating (TSH),‡ and prolactin,* were dissolved in water at *p*H 7. Cortisone acetate (Sharp and Dohme) was suspended in saline and desoxycorticosterone acetate (DOCA) (Schering) was dissolved in sesame oil. Growth hormone and ACTH were injected intraperitoneally whereas the other hormones were given subcutaneously.

Serial blood samples were taken from the tail and analyzed as previously described[7] for glucose,[9] ketone

Presented at the 80th Meeting of the American Physiological Society in Philadelphia April 14-18, 1958.[6]

From the Laboratory of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Maryland.

* The growth hormone (NIH-BGH-1, lot R-50109) and prolactin (NIH-SP-1, lot 10109) were gifts from the Endocrinology Study Section, National Institutes of Health. The growth hormone was prepared from beef pituitaries and prolactin from sheep pituitaries. The prolactin contained 20 I.U. per mg.

† The ACTH (Wilson lot 102201) was prepared from hog pituitaries by the oxycellulose method of Astwood and was a gift from Dr. David Klein of the Wilson Laboratories, Chicago, Illinois. It contained 90 subcutaneous USP units per mg.

‡ The TSH (Lot PC-8-93-D) was prepared from beef pituitaries[8] and was a gift from Dr. Peter Condliffe of the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland. It contained 3.7 USP units per mg.

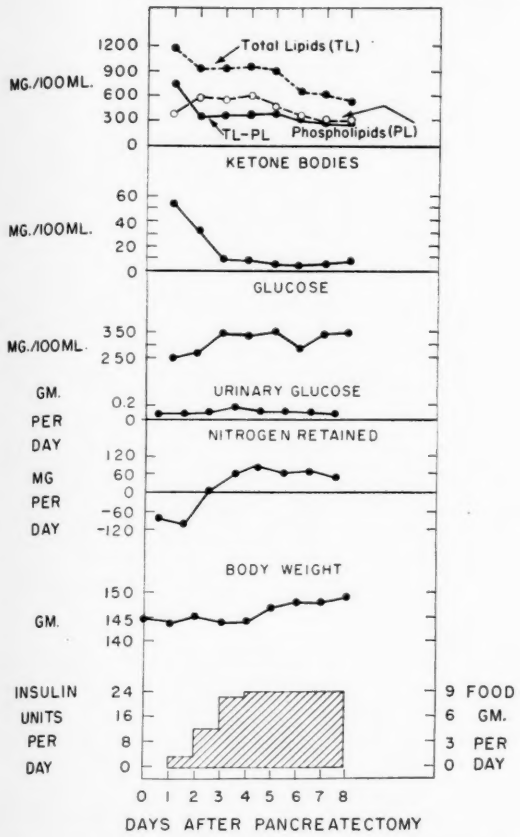


FIG. 1. Effects of insulin and tube-feeding in fourteen pancreatectomized female rats. Approximately 2.7 units of regular insulin were given for each gram of food. The blood levels on the first day were determined twenty hours after pancreatectomy; thereafter they were measured each morning seventeen hours after the last insulin injection and feeding.

bodies,[10] total lipids,[11] and phospholipids.[12] Ketone bodies are expressed in terms of acetone. Since the chief component of the excess fat in the blood of the diabetic rat is triglycerides[7] and since the various treatments in the present study had very little effect on the blood phospholipid levels (table 1), only the values for the nonphospholipid fats (total lipids minus phospholipids) are presented in most of the graphs (figures 3-8). Urine was collected for twenty-four-hour periods under toluene and analyzed for ketone bodies.[7] Total fat and phospholipid contents of liver and kidney were determined by the methods used for blood.[7] The total carbohydrate content of the liver was measured by determining the amount of reducing substance in an aliquot of the tissue homogenate.[13] When the carbohydrate content was

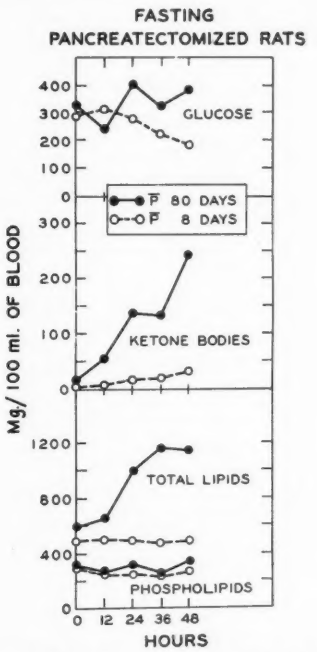


FIG. 2. Response of rats pancreatectomized (P) for eighty days (six rats) and for eight days (seven rats) to insulin withdrawal and fasting. The last insulin injection and feeding were given seventeen hours before the start of the experimental period.

determined the animal was killed by severing the neck, and the liver was immediately removed, weighed, and frozen until analyzed. The fat and nitrogen contents of the carcass were determined by the method of Kochakian and Stettner.[14]

## RESULTS

In a preliminary study observations were made in fourteen pancreatectomized rats while they were being treated with insulin and tube-fed (figure 1). The blood levels were measured each morning before giving insulin. By the third day after pancreatectomy the morning blood ketone bodies and nonphospholipid fats (TL-PL) were at low and constant levels, about 9 and 300 mg. per cent respectively, whereas the phospholipids increased to 600 mg. per cent for several days and then decreased by the seventh day to 300 mg. per cent. Although the morning blood glucose was always elevated, glycosuria was small, less than 3 per cent of the carbohydrate fed. With this regimen of insulin administration and feeding, the pancreatectomized rats retained nitrogen and gained about 5 gm. of body weight during the first eight postoperative days.

When insulin and food were withheld, rats pancre-

TABLE 1

Effects of different hormones on blood lipids and urinary excretion of ketone bodies in fasting rats[a]

| Group[b] | Dose, mg. per day | No. of rats | Body weight at start of fast, gm. | Survival, days | Change in blood, mg. per cent[d] Phospho-lipids | Total lipids | Ketone bodies | Urinary excretion of ketone bodies, mg./day 1st | 2nd |
|---|---|---|---|---|---|---|---|---|---|
| P-80 | — | 6 | 192 | 1-2 | 33 | 576* | 212* | 65 ± 16 | 76 ± 36 |
| P | — | 7 | 169 | 2 | —28 | 33 | 22* | 0 | 2 ± 2 |
| P-sham A | — | 6 | 170 | 2 | 107 | 526* | 161* | 31 ± 17 | 97 ± 20 |
| AP | — | 5 | 172 | 2 | —8 | —81 | 13* | 0 | 0 |
| AP | — | 5 | 157 | 2 | —34 | —12 | 11* | 0 | 0 |
| HP | — | 7 | 170 | 2 | —17 | —24 | 9* | 0 | 0 |
| HP + GH | 0.3 | 4 | 167 | 2 | —45 | —145* | 8* | 0 | 0 |
| " " | 2.0 | 3 | 167 | 2 | —41 | —100 | 8* | 0 | 0 |
| " " | 5.0 | 5 | 162 | 2 | —102 | —33 | 4 | 0 | 0 |
| HP + Cortisone | 0.25 | 3 | 156 | 2 | —63* | 64 | 37 | 0 | 2 ± 2 |
| " " | 1.0 | 6 | 153 | 2 | —26 | 188 | 58* | 0 | 39 ± 21 |
| " " | 2.0 | 5 | 165 | 1 | 90 | 649 | 104* | 43 ± 10 | — |
| " " | 4.0 | 5 | 167 | 1 | —5 | 302 | 151* | 67 ± 16 | — |
| AP + Cortisone | 2.0 | 5 | 169 | 2 | 111 | 895* | 150* | 57 ± 26 | 149 ± 20 |
| AP + DOCA | 3.0 | 6 | 159 | 2 | —16 | 1 | 24* | 3 ± 3 | 1 ± 1 |
| H + Cortisone | 4.0 | 8 | 169 | 1[c] | —28 | —75 | 2 | 0 | — |
| N + Cortisone | 4.0 | 6 | 169 | 1[c] | —14 | —25 | 5* | 0 | — |
| HP + ACTH | 0.3-0.4 | 5 | 163 | 2 | 8 | —41 | 117* | 55 ± 12 | 230 ± 20 |
| AP + ACTH | 0.3-0.4 | 6 | 160 | 2 | 52 | —13 | 3 | 0 | — |
| H | — | 3 | 162 | 2 | —17 | —31 | 12* | 0 | 0 |
| H + ACTH | 0.3-0.4 | 6 | 163 | 2 | —20* | —42 | 4* | 0 | 4 ± 4 |
| HP + TSH | 1.2 | 7 | 177 | 2 | —36 | 29 | 17* | 4 ± 4 | 8 ± 8 |
| HP + Prolactin | 8.0 | 4 | 157 | 2 | —92* | —148* | 10* | 1 ± 1 | 1 ± 1 |

[a] Mean ± standard error. Experimental period was begun seventeen hours after the last insulin injection and the last feeding.

[b] P = pancreatectomized; A = adrenalectomized; H = hypophysectomized; N = normals. All pancreatectomies were made seven to twelve days before start of fast except in group P-80 in which the pancreatectomies were made eighty days before. All adrenalectomies and hypophysectomies were made at the start of the experimental period. Complete removal of the whole pituitary gland, as evidenced by absence of pituitary tissue in or near the sella turcica when examined at autopsy with 10x magnification, was achieved in all hypophysectomized rats presented in this report.

[c] Killed at twenty hours.

[d] Difference between initial and final concentrations.

*Designates the statistically significant differences ($p<0.5$).

atectomized for eight days did not develop ketosis (figure 2). During the two-day experimental period there was an increase of only 22 mg. per cent in the blood ketone body level, no change in blood total fats and phospholipids, and a small decrease in the blood glucose level. However, when rats maintained for eighty days after pancreatectomy were deprived of insulin, they immediately developed severe ketosis and died within sixty-five hours; half of them were dead within forty-two hours. The terminal blood level of ketone bodies averaged 230 mg. per cent and that of total lipids, nearly 1,200 mg. per cent (figure 2). The blood ketone bodies were significantly elevated, to 20 mg. per cent, as early as seventeen hours after the last insulin injection and tube-feeding, the onset of the experimental period. There was no change in blood phospholipids and only a small increase in blood glucose during the experimental period.

Subsequent experiments were performed seven to twelve days after pancreatectomy. As noted above, withdrawal of insulin and food did not produce ketosis in these animals during the two-day period (figure 3). However, when such rats were subjected to sham adrenalectomy at the onset of the experimental period, all developed severe ketosis. At forty-eight hours, the ketone bodies in the blood exceeded 150 mg. per cent and the nonphospholipid fats were increased 350 mg. per cent. The blood glucose level rose during the second day. The dependence of this response on an intact pituitary-adrenal axis is illustrated on the right side of figure 3.

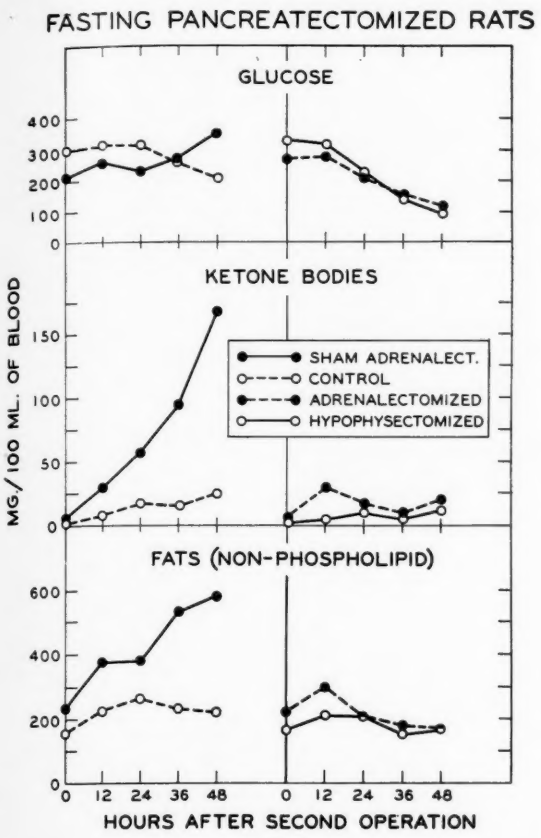


FIG. 3. Effect of sham adrenalectomy, adrenalectomy, and hypophysectomy on rats pancreatectomized for about ten days. There were six to eight rats in each group.

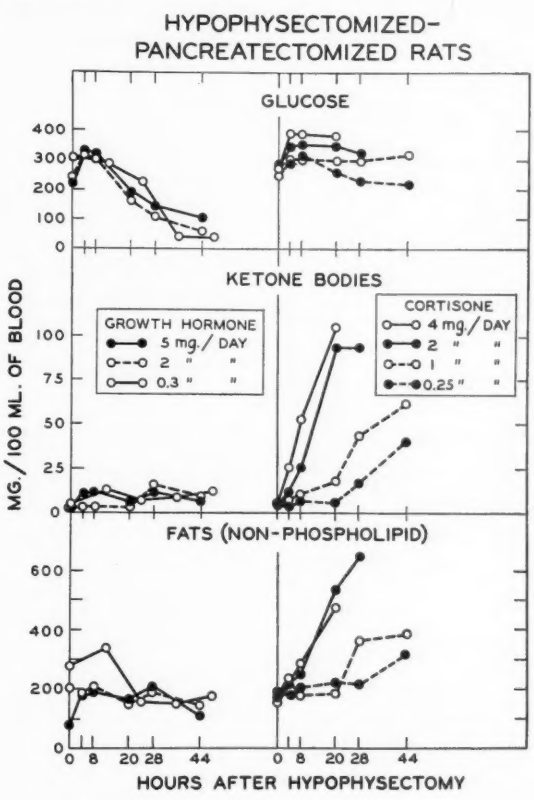


FIG. 4. Effect of different amounts of growth hormone and cortisone, given separately, on hypophysectomized-pancreatectomized rats. The hormones were given at one and twenty hours after hypophysectomy. The number of rats in each group, which ranged from three to six, is indicated in table 1.

Either adrenalectomy or hypophysectomy prevented the response to the operative stress. There was, however, a small transient rise in blood ketone bodies and fats in the adrenalectomized rats during the first twelve hours. The fall in blood glucose in the animals that were doubly operated upon was related to the depletion of liver glycogen.

At the time of the second operation the liver glycogen exceeded 7 per cent whereas forty-eight hours later it was less than 0.5 per cent (table 2).

Administration of growth hormone to hypophysectomized-pancreatectomized rats had very little effect on the blood ketone bodies and no effect on the blood glucose and lipids (figure 4). Even large doses, 5 mg./day, increased the blood ketone bodies only 8 mg. per cent.

In insulin-deficient hypophysectomized rats cortisone had a pronounced ketogenic action (figure 4). Four milligrams of cortisone killed all animals within twenty-four hours and 2 mg./day killed half of them within forty-eight hours. The elevated levels of blood ketone bodies were associated with marked increases in blood lipids. The well-known gluconeogenic action of cortisone was reflected in the sustained hyperglycemia of these animals. Note the dose-response relationship between cortisone and the levels of the blood constituents studied. Even 0.25 mg. of cortisone per day produced a significant ketogenic effect in two thirds of the animals.

Cortisone was also ketogenic in adrenalectomized-pancreatectomized rats whereas desoxycorticosterone acetate had no effect on the ketone body level in blood (figure 5). Cortisone, 2 mg./day, increased the blood level of ketone bodies to 150 mg. per cent and of non-

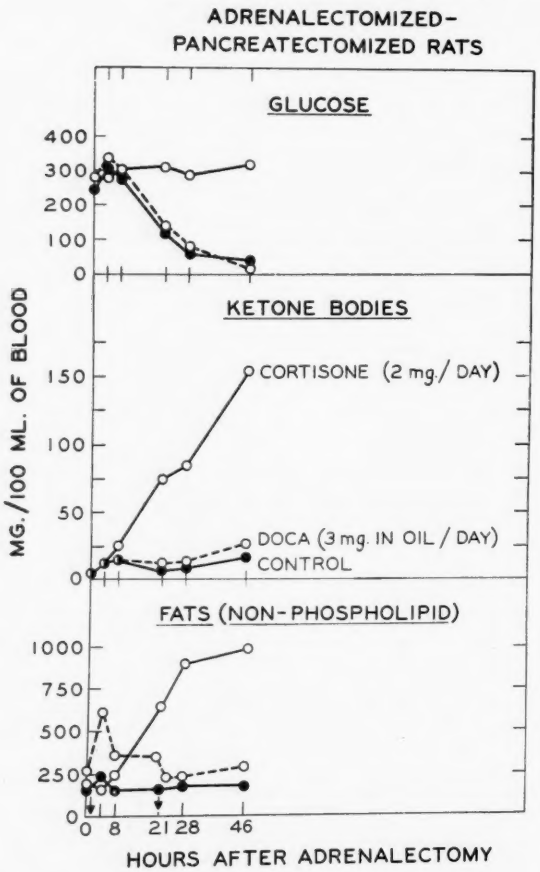


FIG. 5. Effect of cortisone and DOCA on adrenalectomized-pancreatectomized rats. The hormones were given at one and twenty-one hours after adrenalectomy. There were five or six rats in each group.

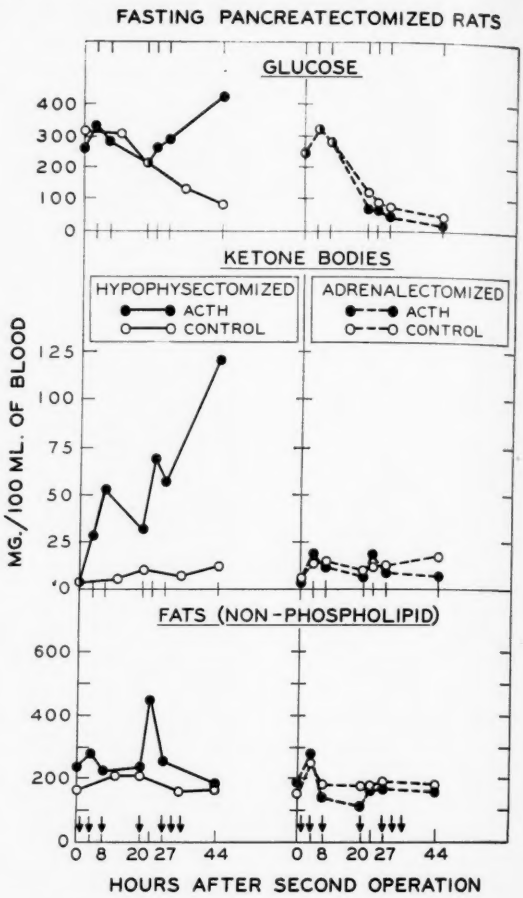


FIG. 6. Effect of ACTH on pancreatectomized rats deprived of either the pituitary or adrenal glands. ACTH, 0.1 mg., was given at times indicated by the arrows. There were six rats in each group.

phospholipid fats to 1,000 mg. per cent, and maintained the blood glucose at the initial level of 300 mg. per cent. DOCA had no effect on the blood glucose level. There was, however, a transient rise in the blood non-phospholipid fats following the first injection of DOCA but this may have been due to the large amount of oil, 600 mg., in which the hormone was suspended.

Administration of ACTH three to four times daily also produced a marked elevation in the blood ketone body concentration of hypophysectomized-pancreatectomized rats (figure 6). The hyperketonemia, which reached a maximum of 120 mg. per cent, was accompanied by a transient elevation of blood fats on the second day.

The fluctuations in blood ketone body levels probably reflect the short life of the hormone. During the second day there was a marked rise in the blood glucose level. The data on the right side of figure 6 demonstrate that the adrenal glands are necessary for these effects of ACTH.

Cortisone and ACTH had no effect on the blood ketone bodies or blood lipids in hypophysectomized rats with an intact pancreas and in normal rats (figure 7). There was also no effect on the blood glucose level during the first day. During the second day the hypophysectomized rats treated with ACTH had a small increase in blood glucose which was associated with a small drop in the blood ketone body level. (The animals treated with cortisone were fed ad libitum until seventeen hours before the onset of the experimental period. The ani-

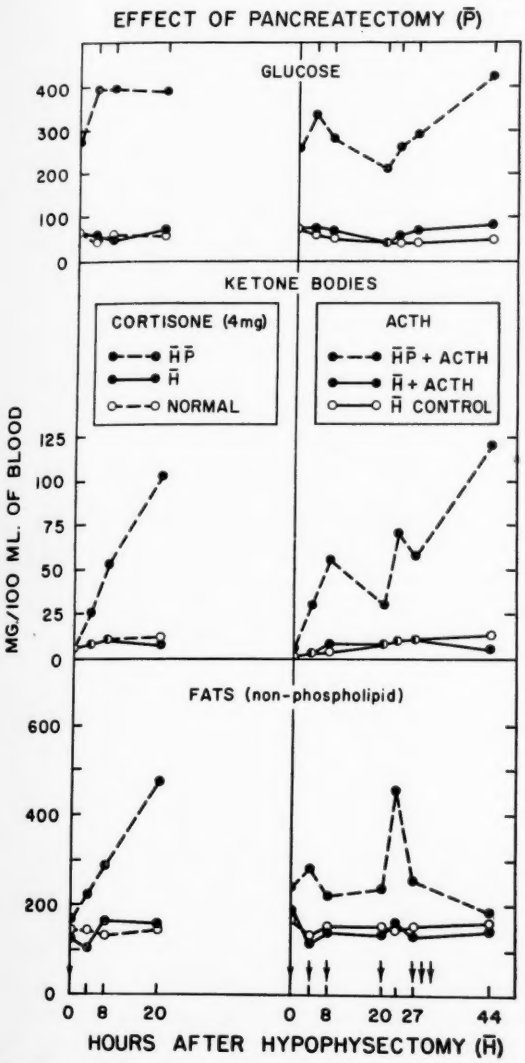


FIG. 7. Effect of pancreatectomy on response to cortisone and ACTH injections. The hormones were injected at times indicated by the arrows. The dosage of cortisone was 4 mg. and of ACTH, 0.1 mg./injection. There were five to eight rats in each group except in the hypophysectomized control group which had only three rats.

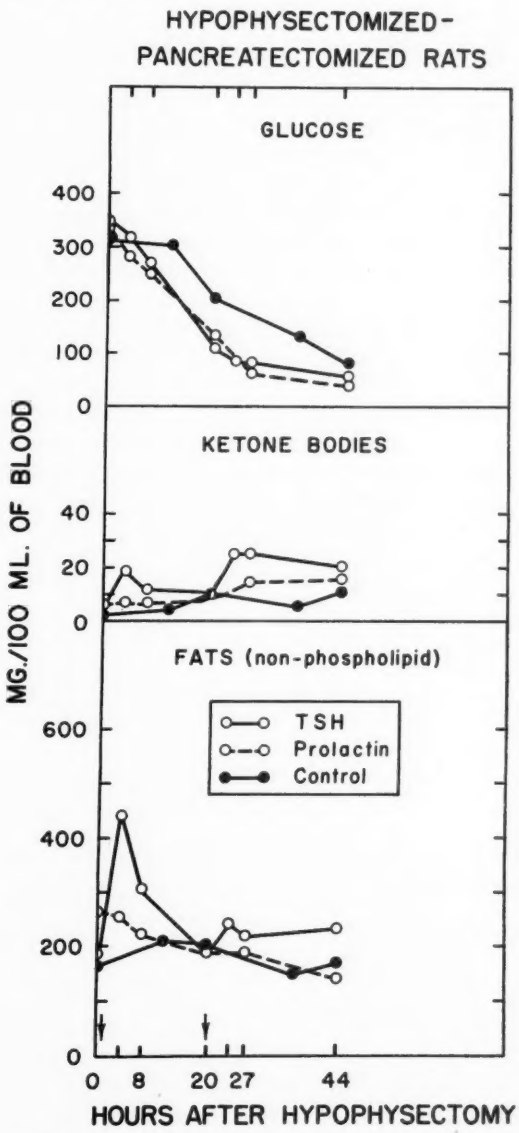


FIG. 8. Effect of TSH and prolactin on hypophysectomized-pancreatectomized rats. The hormones, 1.2 mg. TSH and 8 mg. prolactin, were given at one and twenty hours after hypophysectomy. There were four rats in the prolactin group and seven in each of the other groups.

mals treated with ACTH were tube-fed for eight days in the same manner as the pancreatectomized rats and received their last feeding seventeen hours before the start of the experimental period.)

TSH increased the blood level of ketone bodies 15 mg. per cent in hypophysectomized-pancreatectomized rats within three hours after each injection (figure 8); the maximal level, however, did not exceed 25 mg. per cent. After the first, but not the second, injection of TSH, there was a transient increase in the blood concentration of nonphospholipid fats. TSH had no significant effect on the blood glucose level.

Prolactin in very large doses did not affect the blood glucose, ketone bodies, or lipids in hypophysectomized-

pancreatectomized rats (figure 8).

Table 1 presents the effects of the various treatments on the blood lipids and ketone bodies in all groups studied. There was very little change in the blood phospholipids: In some groups they increased 100 mg. per cent but they never made up more than 25 per cent of the gain in total lipids; in others they decreased as the total lipids increased. The changes in blood lipids and ketone bodies did not appear to be closely related.

Ketonuria occurred only in those rats with terminal blood ketone body levels of more than 60 mg. per cent (table 1). In general, ketone bodies were not found in the urine of rats unless the blood level exceeded 25-30 mg. per cent.

The amounts of carbohydrate in the liver and of fat in the liver and kidneys of all groups at the end of the experimental period are presented in table 2. Since the amount of phospholipids in these tissues changed very little, the amount of nonphospholipid fats was calculated by difference (total lipids minus phospholipids) in order to obtain a better idea of the magnitude of change in the triglycerides in these tissues.

The carbohydrate content of the liver at the end of the experimental period was less than 25 mg. in all animals doubly operated upon except those given ACTH when the adrenals were intact or given cortisone (table

TABLE 2

Effects of different hormones on lipid and carbohydrate content of tissues of fasting rats[a]

| Group[b] | Dose mg. per day[c] | No. of rats | Hrs. after start of experiment[d] | Liver | | | | Final blood ketone bodies mg. per cent | Kidneys | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Weight gm. | Total CHO mg.[e] | Total fats mg. | Nonphospholipid fats mg. | | Weight gm. | Total fats mg. | Nonphospholipid fats mg. |
| N-4 | — | 3 | 0 | 5.7±0.2 | 215±17 | 210±14 | 45±9 | 1 | 1.03±.04 | 36±5 | 9±3 |
| N-ad libitum | — | 6 | 24 | 4.9±0.2 | 11±1 | 249±37 | 76±28 | 10 | — | — | — |
| P-4 | — | 7 | 0 | 8.5±0.5 | 631±51 | 230±19 | 39±10 | 2 | 1.12±.04 | 41±3 | 8±2 |
| P-80 | — | 6 | 24-48 | 6.2±0.5 | — | 1,000±263 | 837±259 | 230 | 1.80±.15 | 124±16 | 80±15 |
| P | — | 7 | 48 | 5.1±0.3 | 53±18 | 164±11 | 26±13 | 25 | 1.20±.04 | 40±2 | 12±2 |
| P-sham A | — | 6 | 48 | 5.6±0.3 | — | 454±88 | 328±81 | 167 | 1.40±.03 | 79±7 | 42±8 |
| AP | — | 5 | 48 | 4.7±0.2 | — | 133±8 | 24±7 | 21 | 1.30±.07 | 47±1 | 12±1 |
| AP | — | 5 | 48 | 4.2±0.1 | 15±4 | 144±5 | 9±4 | 16 | 1.05±.02 | 36±1 | 5±2 |
| HP | — | 7 | 48 | 4.7±0.4 | 20±8(4) | 153±12 | 23±3 | 12 | 1.10±.08 | 42±2 | 12±1 |
| HP + GH | 0.3 | 4 | 48 | 4.5±0.4 | 16±6(2) | 168±22 | 31±8 | 14 | 1.10±.03 | 41±4 | 12±3 |
| " " | 2.0 | 3 | 48 | 4.1±0.1 | 12±6 | 126±6 | 12±3 | 11 | 1.00±.02 | 34±1 | 7±1 |
| " " | 5.0 | 4 | 48 | 4.4±0.3 | 19±11 | 153±28 | 38±19 | 7 | 1.14±.05 | 42±4 | 12±2 |
| HP + Cortisone | 0.25 | 3 | 48 | 4.7±0.1 | 47±18 | 171±11 | 18±28 | 41 | 1.07±.04 | 41±4 | 10±5 |
| " " | 1.0 | 6 | 48 | 4.5±0.2 | 82±7 | 174±22 | 52±24 | 61 | 1.10±.08 | 47±7 | 18±6 |
| " " | 2.0 | 5 | 28 | 4.8±0.2 | 66±9 | 281±29 | 144±25 | 108 | 1.40±.04 | 55±3 | 26±2 |
| " " | 4.0 | 5 | 20 | 5.2±0.5 | 90±7 | 257±42 | 135±37 | 122 | 1.13±.02 | 60±2 | 32±2 |
| AP + Cortisone | 2.0 | 5 | 48 | 5.8±0.3 | 62±5 | 630±135 | 434±141 | 155 | 1.35±.06 | 92±9 | 50±10 |
| AP + DOCA | 3.0 | 5 | 48 | 4.7±0.2 | 11±2 | 176±16 | 47±9 | 28 | 1.18±.04 | 43±2 | 12±1 |
| H + Cortisone | 4.0 | 4 | 24 | 4.9±0.2 | 42±18 | 309±41 | 130±39 | 8 | 1.23±.05 | 44±5 | 10±1 |
| N + Cortisone | 4.0 | 3 | 24 | 5.3±0.5 | 69±18 | 254±14 | 66±8 | 11 | 1.17±.07 | 34±5 | 2±6 |
| HP + ACTH | 0.3-0.4 | 5 | 48 | 4.8±0.3 | 53±10 | 254±87 | 117±81 | 120 | 1.21±.05 | 63±8 | 28±7 |
| AP + ACTH | 0.3-0.4 | 6 | 48 | 4.2±0.2 | 10±2 | 135±6 | 6±3 | 7 | 1.13±.04 | 42±3 | 9±2 |
| H | — | 3 | 48 | 4.1±0.1 | 9±1 | 157±7 | 29±7 | 13 | .99±.06 | 33±1 | 8±1 |
| H + ACTH | 0.3-0.4 | 6 | 48 | 4.3±0.1 | 50±8 | 211±19 | 69±19 | 5 | 1.00±.03 | 36±2 | 10±2 |
| HP + TSH | 1.2 | 7 | 48 | 4.5±0.2 | 13±6 | 141±8 | 32±8 | 20 | 1.11±.03 | 37±3 | 12±2 |
| HP + Prolactin | 8.0 | 4 | 48 | 4.2±0.3 | 13±3 | 138±7 | 29±3 | 16 | 1.08±.06 | 39±5 | 10±2 |

[a] Mean ± standard error.
[b] N-4, normals tube-fed for four days the same amounts of food as given to Group P-4; N-ad libitum, normals fed ad libitum, food cups removed from cage seventeen hours before start of experimental period; P, pancreatectomized for seven to twelve days at the start of experiment; P-4 and P-80, pancreatectomized for four and eighty days, respectively; A, adrenalectomized; H, hypophysectomized.
[c] See text for schedule of injections.
[d] Experimental period begun seventeen hours after last insulin injection and last feeding.
[e] Figure in parentheses indicates number of animals measured if less than that used for other determinations.

2). The treated rats had 40-90 mg. of carbohydrate in the liver. The terminal blood glucose level in the diabetic rats was closely related to the amount of carbohydrate in the liver.

The fat content of the liver was elevated in fasting rats pancreatectomized for seven to twelve days only when they had been subjected to sham adrenalectomy, or given ACTH when the adrenals were intact, or given 2-4 mg. of cortisone. None of the other hormonal treatments had any significant effect on the fat content of the liver. Cortisone and ACTH produced a mild adipokinetic effect in hypophysectomized rats with an intact pancreas. Rats pancreatectomized for eighty days accumulated very large amounts of fat in the liver when fasted. Excess deposition of fat in the liver was always accompanied by accumulation of fat in the kidneys.

In the diabetic rats there was a very close relationship between the amount of nonphospholipid fats in the liver and the final blood ketone body level (table 2 and figure 9). Such was not the case in rats with an intact pancreas.

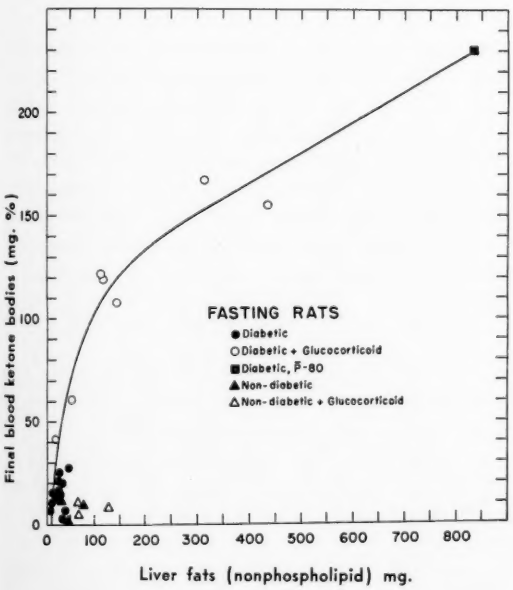


FIG. 9. Relationship between final blood ketone body level and amount of nonphospholipid fat in the liver. The glucocorticoid groups include those animals given ACTH with the adrenal glands intact or cortisone, or subjected to sham adrenalectomy. There were three to seven animals for each point (see table 2). All diabetic rats except those in group P-80 were pancreatectomized for seven to twelve days; those in group P-80 were pancreatectomized for eighty days.

## DISCUSSION

Increased fat mobilization and ketosis did not develop in rats pancreatectomized for seven to twelve days when deprived of insulin unless adrenocortical secretion was stimulated or cortisone was given. ACTH was the only pituitary hormone necessary for this response. Hyperglycemia was maintained only in those animals that developed ketosis. Since DOCA had no effect, the adrenal hormones responsible for the mobilization of fat and for ketosis in the diabetic are undoubtedly glucocorticoids. These findings support the hypothesis first proposed by Long and Lukens that the ameliorative effect of hypophysectomy on diabetes is due primarily to suppression of adrenal cortical secretions.[2]

When rats pancreatectomized for eighty days were deprived of insulin they rapidly developed severe ketosis and died in coma without external stimulus. Hypophysectomy prevented the above (unpublished observations). One possible explanation for the difference between the eight-day and eighty-day diabetics is that the pituitary-adrenal axis was more sensitive to the metabolic changes associated with insulin withdrawal in the animals that had a longer time to recover from the operative trauma associated with pancreatectomy. Another possibility is that the large stores of body fat in the older diabetics may require less glucocorticoids for fat release during the insulin-deficient state.* It was recently observed that the degree of fat mobilization and ketosis following pancreatectomy in the rat was closely related to the amount of body fat stores.[7]

The relationship between the final blood ketone body level and the amount of nonphospholipid fat in the liver is summarized in figure 9. Since the ketonemia in all groups with ketosis was still rising at the end of the experimental period, and since little is known about the effects of the various factors studied on ketone body utilization in the diabetic, it is assumed that the blood level reflects the rate of ketogenesis. From the foregoing it would appear that there is a close relationship in the diabetic rat between the rate of ketone body formation and the amount of triglycerides in the liver. This relationship has been seen also in preliminary studies with liver slices (unpublished observations). The findings presented in figure 9 suggest that in the diabetic glucocorticoids increase the amount of ketone bodies formed primarily by mobilizing fat to the liver and, also, by

* The rats pancreatectomized for eighty days had 12.6 ± 2.4 (standard error) gm. of fat and 5.4 ± 0.2 gm. of nitrogen in the carcass at the end of the experimental period; those pancreatectomized for eight days had only 5.6 ± 0.6 gm. of fat and 4.8 ± 0.2 gm. of nitrogen.

accelerating the rate of ketogenesis for a given liver fat content. A direct effect of glucocorticoids on adipose tissue is suggested by the rapid rise in blood lipid levels after cortisone administration (figures 4 and 5). The presence of an intact pancreas prevents these effects of glucocorticoids on fat release, hepatic accumulation of fat, and on ketogenesis.

The ketogenic action of cortisone and of ACTH has been observed also in diabetic humans and requires relatively small amounts of hormone. Balfour and Sprague[15] found that administration of 2-4 mg. of cortisone produced severe ketosis in two diabetic patients with Addison's disease when insulin and food were withheld; DOCA had no effect. Kinsell and his associates[16] reported a severely diabetic patient who developed ketosis over a period of three days when fasted and deprived of insulin. Under similar conditions but given ACTH, she developed severe ketosis within thirty hours, with a maximal blood ketone body level of 120 mg. per cent, and required several hundred units of insulin for control of ketosis. The hyperketonemia in this patient was accompanied by a pronounced rise in the blood glucose level.

Gilman and his associates[17,18] have observed that cortisone has an adipokinetic action in hypophysectomized-pancreatectomized baboons which is prevented by insulin administration. There was no effect of cortisone on the blood ketone bodies in the few animals studied.

Several workers have reported that the level of corticosteroids in the urine is greatly elevated during diabetic coma.[19-21] They concluded that the increased adrenal cortical activity was the result and not the cause of the ketosis. This conclusion is not compatible with (1) the classical observations that hypophysectomy or adrenalectomy prevents the development of diabetic ketosis;[1,2] (2) that adrenal stimulation is necessary for development of coma in young diabetic rats (figures 3 and 6); (3) that ketosis develops in diabetic humans and rats with adrenal insufficiency when they are injected with cortisone[15] (figure 5). It must be noted that the ketogenic action of small amounts of glucocorticoids is manifested only when there is a severe insulin deficiency. Ketosis has been produced by cortisone administration in diabetic patients receiving insulin[22] and in intact rats[23] but only when large amounts of the hormone and a high fat diet were given. On the other hand, the hyperketonemia of fasting is suppressed by administration of glucocorticoids and ACTH if the pancreas is intact.[16,24]

Recent reports have claimed that the ketogenic effect of ACTH is not mediated through the adrenal gland.[25,26] These conclusions, which were drawn from studies in nondiabetic animals, are opposite to those presented here. In many studies the criterion for a ketogenic effect of a hormone has been an elevation of blood ketone bodies within three hours of less than 10 mg. per cent and often less than 5 mg. per cent. Since the accumulation of ketone bodies in the blood is the resultant of many reactions and is affected by factors other than endocrine, interpretation of such small changes in blood level is difficult.

There have been numerous reports that growth hormone and thyroid stimulating hormone are ketogenic.[27-30] These studies were made in nondiabetic animals and, as discussed above, the ketogenic effects were most often based on very small elevations of the blood ketone body concentration. In the hypophysectomized diabetic rat very large amounts of growth hormone and of TSH produced an increase in the blood ketone body level of less than 15 mg. per cent (figures 4 and 8). These effects are negligible when compared to the pronounced accumulation of ketone bodies in the blood and urine when ACTH or cortisone was given (figures 4 and 6).

Growth hormone preparations have a pronounced diabetogenic effect in dogs and cats but not in rats.[28] Recent studies in "totally" pancreatectomized rats, with and without the pituitary gland, have demonstrated that growth hormone has no effect on their insulin requirement.[31,32] In the present study growth hormone had no effect on the blood glucose level of the fasting hypophysectomized diabetic rat. Prolactin, which was studied because of the suggestion that it may be one of the diabetogenic factors of the pituitary gland,[33] also had no effect on the blood glucose of the pancreatectomized-hypophysectomized rats.

## SUMMARY

"Totally" pancreatectomized rats were maintained with insulin and tube-fed for seven to twelve days. Seventeen hours after the last insulin injection and meal some of the rats were subjected to either hypophysectomy, adrenalectomy, or sham adrenalectomy. One or two hours later administration of one of the following hormones was begun: growth hormone, adrenocorticotrophic hormone, thyroid stimulating hormone, prolactin, cortisone, or desoxycorticosterone. Pancreatectomized rats developed ketosis only when given cortisone or when the secretion of adrenal hormones was increased by ACTH administration or by operative stress. Hyperglycemia in animals that had been doubly operated upon was maintained only in those that developed ketosis. The ketogenic action of ACTH was mediated via the adrenal

gland. In normal and hypophysectomized rats with an intact pancreas the ketogenic effects of cortisone and ACTH were not manifested.

The final blood ketone body level in the diabetic rats was closely related to the amount of nonphospholipid fats in the liver. It is suggested that in the diabetic the glucocorticoids produce ketosis by mobilizing fat to the liver and by accelerating the rate of ketogenesis by the liver. Glucocorticoids also increased the fat content of the kidneys of diabetic rats.

The results demonstrate that the essential hormonal factors for ketosis in the diabetic rat are severe insulin deficiency and glucocorticoids. These findings support the view first proposed by Long and Lukens that the ameliorative effect of hypophysectomy on diabetes is due primarily to suppression of adrenal cortical secretions.


## SUMMARIO IN INTERLINGUA

*Action Cetogene De Hormones Pituitari E Adrenal In Rattos Pancreatectomisate*

Rattos "totalmente" pancreatectomisate esseva mantenite con insulina e alimentate via tubos durante periodos de inter septe e dece-duo dies. Dece-septe horas post le ultime injection de insulina e le ultime repasto, gruppos de rattos esseva subjicite a hypophysectomia o a adrenalectomia o a adrenalectomia fictitie. Inter un e duo horas plus tarde, le administration de un del sequente hormones esseva comenciate: Hormon de crescentia, hormon adrenocorticotrophic, hormon thyroido-stimulatori, prolactina, cortisona, disoxycorticosterona. Rattos pancreatectomisate disveloppava cetosis solmente quando illos recipeva cortisona o quando le secretion de hormones adrenal esseva augmentate per ACTH o stress operatori. Hyperglycemia in animales duplemente operate esseva mantenite solmente in le casos in que cetosis se disveloppava. Le action cetogene de ACTH esseva mediate via le corpore adrenal. In rattos normal e hypophysectomisate sed non pancreatectomisate, le effecto cetogene de cortisona e ACTH non esseva manifeste.

Le final nivello sanguinee de corpores cetonic in le rattos diabetic esseva intimemente relationate al quantitate de grassias non-phospholipidic in le hepate. Es suggerite que in diabeticos le glucocorticoides produce cetosis per mobilisar le migration de grassia verso le hepate e per accelerar le cetogenese in iste organo. Glucocorticoides etiam augmentava le contento de grassia in le renes de rattos diabetic.

Le resultatos demonstra que le factores hormonal que es essential pro le disveloppamento de cetosis in rattos diabetic es sever grados de carentia de insulina e le presentia de glucocorticoides. Iste constatationes supporta le conception primo formulate per Long e Lukens que le effecto melioratori exercite super diabete per le effectuation de hypophysectomia resulta primarimente del suppression de secretiones adrenocortical.



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The reawakening of the interest of medical geneticists in the biochemical backgrounds of genetic diseases apparently had to await the occurrence of a number of other developments.[1] Three main events brought into sharp focus the steps intervening between the presence of a gene in the cell and the appearance of a trait or disease in the individual, and thus led to a renewed interest in Garrod's suggestions. The first of these events was the firm establishment of the concept that all metabolism proceeds through series of small sequential steps, each step catalyzed by an enzyme. The second was the demonstration[2] that increase in radiation level can increase the mutation rate. The third event was the realization[3] that with the aid of radiation-induced mutations the biochemical activities of genes can be definitively studied in micro-organisms which are peculiarly suited both to genetic and biochemical investigation. The researches undertaken all over the world along these lines have brilliantly demonstrated the facts that many enzyme dysfunctions are indeed referable to specific mutant genes and that it is reasonable to presume that the production of normal enzymes is dependent on the activity of the normal unmutated alleles of these mutant genes.

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*Special Articles*

# Diabetes in Industry

*Leo Wade, M.D., New York City*

In July 1952, the Committee on Employment of the American Diabetes Association published "Suggested Standards for the Employment of Diabetics." This committee of diabeticians sought the advice and guidance of several physicians with knowledge of, and experience in, the application of medicine to problems in industry. The statement is admirable in its simplicity, brevity and forthright presentation of basic principles.

Shortly after publication of the suggested standards, an effort was made to adapt the statement to the particular needs of this Company. A tentative policy statement was submitted to members of the medical staff. It was also sent to five competent diabeticians having a known diversity of view with regard to the control of diabetes. No unanimity of opinion could be reached about a number of basic issues. The tentative statement of policy will not be reproduced here, but a few of the problems encountered will be mentioned.

First, in spite of tremendous strides in the control of diabetes with demonstration in many clinics of a marked diminution in the frequency and severity of complications, there are still many diabetics who fare poorly. It is not possible, therefore, to assure management or insurance underwriters that the willful addition of diabetics to an employee group will not adversely affect sickness and permanent disability costs.

Second, while it is recognized that close cooperation with the employee's physician is desirable, any statement by the physician regarding the suitability of the candidate for the job in question is of limited value. The physician is not acquainted with the demands of the job and must ultimately base his recommendations upon what the worker tells him. This is likely to reflect the patient's eagerness for the job, rather than objective facts. To the physician in industry, on the other hand, is delegated full responsibility for the safe assignment of workers. Discharge of this responsibility requires independent judgment and control.

Third, agreement upon criteria for the adequacy of diabetes control was totally lacking. The comments of Soskin regarding this problem are of interest.[5] Inasmuch as many physicians located in widely separated areas are involved, any attempts to establish criteria (either liberal or conservative) lead to strained relations between the physician in industry and the worker's doctor. This is not in keeping with the patient's best interests.

Fourth, it was agreed that workers requiring some dosage of insulin should be restricted from situations in which hypoglycemic episodes might endanger the worker or his confreres. Some thought such restrictions were important if the worker took any insulin, while others thought 40 units did not necessitate restrictions.

Finally, the physical demands made of workers and the conditions of work vary tremendously from one industry to another and even widely within one and the same industry. In petroleum refining, for example, plants must be kept operating on an "around-the-clock" basis 365 days per year. Shift work is an integral part of the working situation. A surprisingly large percentage of workers engage in maintenance operations—almost all crafts and trades are represented. The operation of heavy mechanical equipment and the use of a fabulous variety of tools are required. With rare exceptions, new recruits for employment begin at a uniform level. Progression is by way of fairly stereotyped channels which require a wide variety of physical capacities and abilities. It is not in the interest of the individual worker, his fellow employees, or management to begin this career progression with physical limitations that preclude the worker's traveling a reasonable portion of the course. Management is probably unwise, under such circumstances, to choose for employment an individual with "uncorrectible physical defects or disorders" if a reasonably adequate candidate is available without such limitations.

Dublin and Marks[8] have pointed out that "the problem of diabetes in industry stems not from the applicant for employment, but from the employee who has been in service for a long period." Inasmuch as a number of employees become diabetic during the course of employment, it was decided to review the experience with these workers, hoping thereby to clarify some of the problems.

The employee population studied consisted of approximately 27,000 workers (predominantly white males)

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scattered over some twenty states east of the Mississippi River. Diabetic employees coming to the attention of the examining physicians were the subject of a special brief evaluation for the purpose of this report. No attempt was made to evaluate retroactively former employees who were deceased or on annuitant status at the time of this study.

## PREVALENCE OF DIABETES

Data were collected from each of the Company locations with full-time medical services.* In table 1, the number of known cases in each unit is compared with the employee population at midyear (1956).

The percentage of the employee group with diabetes is approximately proportional to the average age of employees in the several groups. The correlation is not exact since the average age of any two groups might be similar in spite of differences in the age distribution within two such groups. This interpretation is confirmed by the data in table 2. The over-all prevalence of 1.3 per cent seems reasonable in view of the fact that this is a select group, each of whom may be assumed to have had no major defects at the time of employment.

## WHEN AND HOW DIAGNOSIS WAS ESTABLISHED

Some previously unrecognized diabetics were discovered in each age group during the course of employment. Most cases, however, were recognized after thirty-five years of age, with a definite peak of incidence in the forty-five- to sixty-year age group. (See table 3.)†

Four out of five were devoid of symptoms or physical signs of diabetes at the time of discovery. Most of these were recognized in the course of periodic health examinations (66.0 per cent), usually by the plant physician, while 13.3 per cent were found during the course of treatment (usually in a hospital) for other and unrelated medical problems (fractures, hernia repairs, etc.).

Visual disturbances, weight loss, polydipsia, polyuria, caused 18.4 per cent to seek medical advice either in the plant dispensary or from their family physicians. Thus, only one in five was brought to the attention of the physician; he found four of every five cases by the simple routine of urinalysis, later confirmed by blood sugar studies. Inasmuch as such examinations are usually on an annual or biennial basis, it seems improbable that many of the asymptomatic cases were of long standing. The detailed diagnostic studies outlined by Sisk et al.[4] are not practicable when dealing with a large employee population.

One might ask if any clues could arouse suspicion,

TABLE 1

Prevalence of diabetes according to average age and location

| | Male employee population 6/30/57 | Number of diabetics | Per cent of diabetics | Average age |
|---|---|---|---|---|
| Everett Refinery (Massachusetts) | 507 | 13 | 2.6 | 45.2 |
| New Jersey Marketing (Elizabeth, N.J.) | 900 | 14 | 1.6 | 44.5 |
| Charleston Refinery (South Carolina) | 66 | 1 | 1.5 | 44.5 |
| Baton Rouge Refinery (Louisiana) | 6,899 | 86 | 1.3 | 43.9 |
| Bayonne Refinery (Bayonne, N.J.) | 1,835 | 41 | 2.2 | 43.6 |
| Bayway Refinery (Elizabeth, N.J.) | 3,958 | 35 | 0.9 | 42.2 |
| New York Marketing | 790 | 6 | 0.8 | 41.4 |
| Del.—Md.—D.C. Marketing | 806 | 11 | 1.4 | 41.1 |
| New York "15," New York City | 1,074 | 26 | 2.4 | 41.0 |
| Louisiana Marketing | 371 | 4 | 1.1 | 40.9 |
| North Carolina Marketing | 635 | 6 | 0.9 | 40.8 |
| Baltimore Refinery (Maryland) | 1,374 | 11 | 0.8 | 40.0 |
| Virginia—West Virginia Marketing | 815 | 10 | 1.2 | 39.6 |
| South Carolina Marketing | 436 | 2 | 0.5 | 38.2 |
| Totals | 20,466 | 266 | 1.3 | 42.6 |

TABLE 2

Prevalence of diabetes according to age

| Age | Male employee population 6/30/56 | Number of diabetics | Per cent of diabetics |
|---|---|---|---|
| 15 to 20 | 30 | 0 | — |
| 20 to 25 | 506 | 2 | 0.4 |
| 25 to 30 | 1,829 | 4 | 0.2 |
| 30 to 35 | 3,191 | 7 | 0.2 |
| 35 to 40 | 3,366 | 18 | 0.5 |
| 40 to 45 | 2,924 | 26 | 0.9 |
| 45 to 50 | 2,594 | 42 | 1.6 |
| 50 to 55 | 2,444 | 54 | 2.2 |
| 55 to 60 | 2,208 | 63 | 2.9 |
| 60 to 65 | 1,374 | 50 | 3.6 |
| Totals | 20,466 | 266 | 1.3 |

*The author wishes to acknowledge with gratitude the cooperation of these physicians.

† Table 3 and subsequent tables provide data on 315 cases. These additional cases were females, nonwhites and annuitants not included in the previous tabulation.

TABLE 3

When and how diagnosis was established

| Age when discovered | Number | Per cent | Asymptomatic | | | Symptomatic | Unknown |
|---|---|---|---|---|---|---|---|
| | | | Periodic examination by company physician Per cent | Armed services, insurance, and other Per cent | In connection with other illness Per cent | Per cent | Per cent |
| 15-20 | 2 | 0.6 | 0 | 1 | 0 | 1 | 0 |
| 20-25 | 4 | 1.3 | 0 | 3 | 0 | 1 | 0 |
| 25-30 | 9 | 2.9 | 3 | 1 | 1 | 4 | 0 |
| 30-35 | 20 | 6.3 | 11 | 2 | 5 | 2 | 0 |
| 35-40 | 35 | 11.1 | 23 | 2 | 3 | 7 | 0 |
| 40-45 | 37 | 11.8 | 21 | 3 | 2 | 11 | 0 |
| 45-50 | 69 | 21.9 | 44 | 5 | 10 | 9 | 1 |
| 50-55 | 59 | 18.7 | 26 | 4 | 12 | 13 | 4 |
| 55-60 | 52 | 16.5 | 38 | 0 | 5 | 9 | 0 |
| 60-65 | 26 | 8.3 | 19 | 2 | 4 | 1 | 0 |
| Unknown | 2 | 0.6 | 0 | 0 | 0 | 0 | 2 |
| Totals | 315 | 100.0 | 58.8 | 7.3 | 13.3 | 18.4 | 2.2 |

permitting earlier recognition of the disease. In this regard, the family history was examined in each instance. Only slightly more than one fourth of the total group (27.9 per cent) had definite knowledge of other cases in their immediate family. (See table 4.)

In these instances, the parents (51.1 per cent), grandparents (9.1 per cent), and aunts and uncles (11.4 per cent) accounted for roughly three quarters of the relatives having the disease. (See table 5.)

## THE CLINICAL COURSE OF THE DISEASE

Some might question whether the finding of these asymptomatic cases made any positive contribution to the subsequent health and welfare of the individuals. Possibly one is dealing with laboratory data of little real clinical importance. While modes of therapy vary considerably, it seemed likely that the need for insulin to bring about or to maintain control could be used as a measure of the clinical severity of the disease. In table 6, it will be noted that approximately three quarters of these employees did require insulin therapy.

The larger percentage of such cases was found in the group without knowledge of other cases in their family. The fact of insulin therapy did not necessarily reflect better control of the disease. (See table 7.) This, of course, is not surprising since many other factors are important in the control of diabetes.

TABLE 4

Was there family history of diabetes?

| | | |
|---|---|---|
| Yes | 88 | 27.9 |
| No | 195 | 61.9 |
| Uncertain | 32 | 10.2 |
| | 315 | 100.0 |

TABLE 5

Which relative had history of diabetes?

| | | Per cent |
|---|---|---|
| Parents | 45 | 51.1 |
| Grandparents | 8 | 9.1 |
| Aunts & uncles | 10 | 11.4 |
| Siblings | 22 | 25.0 |
| Other or unknown | 3 | 3.4 |
| | 88 | 100.0 |

TABLE 6

Was insulin required for control?

| | Yes Per cent | No Per cent |
|---|---|---|
| Positive family history (88) | 59.1 | 40.9 |
| Negative family history (195) | 82.6 | 17.4 |
| Unknown family history (32) | 62.5 | 37.5 |
| | 74.0 | 26.0 |

TABLE 7

How nearly adequate was control of diabetes?

| | Insulin | | No insulin | | All | |
|---|---|---|---|---|---|---|
| | Number | Per cent | Number | Per cent | Number | Per cent |
| Excellent | 26 | 11.2 | 18 | 22.0 | 44 | 14.0 |
| Good | 103 | 44.2 | 27 | 33.0 | 130 | 41.3 |
| Fair | 66 | 28.3 | 19 | 23.0 | 85 | 27.0 |
| Poor | 24 | 10.3 | 9 | 11.0 | 33 | 10.5 |
| Not known | 14 | 6.0 | 9 | 11.0 | 23 | 7.2 |
| | 233 | 100.0 | 82 | 100.0 | 315 | 100.0 |

TABLE 8

Percentage of diabetic employees with cardiovascular-renal disease and obesity*

| Control | Insulin-treated | | No insulin | | All | |
|---|---|---|---|---|---|---|
| | Cardio-vascular-renal Per cent | Obesity Per cent | Cardio-vascular-renal Per cent | Obesity Per cent | Cardio-vascular-renal Per cent | Obesity Per cent |
| Excellent | 15.4 | 30.8 | 22.2 | 75.0 | 18.2 | 25.0 |
| Good | 18.4 | 26.2 | 29.6 | 14.8 | 20.8 | 23.8 |
| Fair | 37.9 | 40.9 | 57.9 | 36.8 | 42.4 | 40.0 |
| Poor | 45.8 | 54.2 | 44.4 | 44.4 | 45.5 | 51.5 |
| Unknown | 21.4 | 14.3 | 55.6 | 33.3 | 34.8 | 21.7 |
| All | 26.6 | 33.0 | 39.0 | 25.6 | 29.8 | 31.1 |

*15 per cent or more in excess of height-weight data provided by Metropolitan Life Insurance Company tables.

The occurrence of cardiovascular-renal disease and/or obesity is of interest. While the numbers are small, it is of interest that cardiovascular disease was roughly 50 per cent more prevalent in those not receiving insulin. (See table 8.) Obesity, on the other hand, was more common among those requiring insulin (33 per cent compared with 25.6 per cent).

## KINDS OF WORK ASSIGNMENTS

In very few instances was the advent of diabetes a cause for job reassignment. The comment was frequently made, however, that if a promotion or reassignment were necessary for managerial reasons, limitations might have to be imposed by the medical department. In table 9 are tabulated the various kinds of jobs in which these workers were productively engaged. No particular significance can be attributed to the actual numbers assigned to various kinds of work since these merely represent the jobs occupied at the time the diabetes was discovered. It is of interest, however, that workers continued satisfactorily in both sedentary jobs and heavy physical labor.

TABLE 9

Job assignments

| | |
|---|---|
| Boilermaker | 8 |
| Carpenter, painter, etc. | 8 |
| Chemist, laboratory technician, etc. | 7 |
| Clerical, office, etc. | 87 |
| Cook, chef, etc. | 2 |
| Electrician, mechanic, etc. | 19 |
| Engineer | 8 |
| Guard | 8 |
| Janitor | 4 |
| Laborer | 66 |
| Machinist | 17 |
| Mason, insulator | 5 |
| Motor tank sales (drivers) | 12 |
| Process operators, stillman | 33 |
| Pipefitter, welder | 16 |
| Plantman | 5 |
| Pumpman | 3 |
| Salesman (drivers) | 7 |
| | 315 |

Approximately 10 per cent of the total group was restricted. (See table 10.)

TABLE 10

Frequency and cause for restrictions

| | | Number | Per cent of diabetics |
|---|---|---|---|
| Diabetes | | 10 | 3.2 |
| Vascular disease | | 23 | 7.3 |
| Coronary insufficiency | 12 | | 3.8 |
| Hypertensive cardiovascular disease | 7 | | 2.2 |
| Arteriosclerosis obliterans | 2 | | 0.6 |
| Cerebral arteriosclerosis | 1 | | 0.3 |
| Cataracts | 1 | | 0.3 |
| | | 33 | 10.5 |

Only ten workers (3.2 per cent) were restricted because of their diabetes. The possibility of insulin reactions required assignment of eight workers in vocations where they were not likely to injure themselves or others if such reactions occurred. The other two could not be adequately controlled during the irregularities of shift work. (See table 11.)

TABLE 11

Nature of restrictions

| | Number |
|---|---|
| No climbing or working at unprotected heights | 5 |
| No driving of company vehicles | 3 |
| No shift work | 2 |
| | 10 |

Most restrictions were imposed for cardiovascular-renal disease. These were the same as in other workers with similar vascular disease but without diabetes.

## MORBIDITY AND MORTALITY

The premature and unexpected loss of an employee's services through death or premature medical retirement is costly to industry. Management is obviously concerned about the acceptance of applicants for career employment posing such a risk unless such applicants provide unusual abilities that warrant the added risk and cost. A review of morbidity and mortality experience is not presented at this time, but such a study is under way. Two obstacles confront one gathering data: (1) The numbers available for study are small; and (2) these workers have received varying degrees of supervision under many different philosophies of control.

Casual observations suggest that diabetes is not an infrequent complication of other disease (particularly vascular disease) which "tips the scale" in favor of premature medical retirement. Diabetes alone, however, is not the cause of such disability or death, in our experience.

## ARE DIABETICS SATISFACTORY EMPLOYEES?

Diabetes is no respecter of persons. It occurs among serious-minded, competent workers and among those who are nonproductive. The occurrence of diabetes has no effect on underlying personal traits. Efforts to relate work proficiency, accident rates, absenteeism, problem drinking, etc., to the diabetic state are not likely to reveal any startling findings. Weaver and Perret, in a detailed study[6] of about one third the cases reported here, found the average time lost due to illness essentially the same as among nondiabetic employees.

## DISCUSSION

A small percentage (1.3 per cent) of ostensibly normal workers developed clinically significant diabetes mellitus. A careful family history might have made possible the exclusion of approximately one fourth (27.9 per cent) of these individuals from employment. Exclusion on this basis would, however, undoubtedly have led to the refusal of employment to many others who did not develop clinically significant diabetes—how many was not determined in this study.

Fortunately, more than three fourths of these cases appeared after forty years of age. Work assignments had usually become stabilized and were not incompatible with the diabetic state. The advent of insulin therapy in a few instances was thought to warrant restriction of individuals from driving Company vehicles or working about heavy or potentially hazardous machinery. A number of conscientious diabetics could probably work safely even under such circumstances. It is a terrific responsibility, however, for any plant physician to approve such assignments. Fortunately, the problem arose surprisingly infrequently. Restriction of work assignment was sometimes necessitated by concomitant vascular disease. In these instances, the restrictions were no different from those imposed on workers with similar vascular disease, but without diabetes.

Much of the data point up the importance of an adequate occupational health program. Early detection of asymptomatic cases was most frequently the result of periodic health examinations. Proper education of the diabetic (as a part of an over-all health education program) is important. Cardiovascular-renal disease was more common among those not receiving insulin even though control seemed adequate at the time of periodic health audits. Obesity was more common among those requiring insulin therapy. The findings and conclusions are remarkably similar to those of Brandaleone and Friedman.[2]

We believe the data do provide useful clues to job placement of younger diabetics seeking employment. Diabetics should not be hired where normal advancement requires rotation through assignments necessitating the operation of motor vehicles, fast-moving or heavy machinery. Nor should shift work be essential in the course of their careers. Failure to assume a fair share of the "graveyard" shift is not looked upon favorably by fellow workers. The desire of most shift workers to rotate at relatively short intervals (one or two weeks) makes an adjustment of eating and insulin schedules complex and incompatible with optimal diabetic control.

On the other hand, diabetes does not preclude productive employment as evidenced by the experience reported in this study. Physicians responsible for the control of young diabetics, however, would do well to counsel them on the choice of careers, keeping in mind the limitations outlined above.

## CONCLUSIONS

1. Diabetes was present among 266, or 1.3 per cent, of approximately 20,000 petroleum workers.

2. The diagnosis was more common in the older age groups.

3. Approximately 80 per cent of the cases were asymptomatic when discovered.

4. A family history of diabetes was elicited in only 27.9 per cent of these workers.

5. In 74 per cent of the cases, insulin therapy was required for control.

6. In 55.3 per cent of the cases, control was considered "good" or "excellent" while 37.5 per cent were only "fair" or "poor."

7. Clinically significant cardiovascular-renal disease was present in 29.8 per cent of the workers (26.6 per cent of those receiving insulin and 39.0 per cent of those not receiving insulin).

8. Obesity (15 per cent or more in excess of Metropolitan Life Insurance Company standards) was present in 31.1 per cent of the cases (33.0 per cent of those on insulin therapy and 25.6 per cent of the remaining group).

9. A wide variety of job assignments was carried on satisfactorily by these workers.

10. Restrictions because of the diabetes were limited to ten workers (3.2 per cent of the total group) receiving insulin. These restrictions usually precluded the driving of Company vehicles and the operation of fast-moving or heavy machinery.

11. Restrictions because of concomitant cardiovascular-renal disease were more common (twenty-three employees, or 7.3 per cent of the group). These restrictions were the same as in instances of cardiovascular-renal disease not complicated by diabetes.

12. Those responsible for the care of young diabetics might well counsel them to prepare for vocations where the operation of machines, shift work, etc., is not important at the time of employment or during the subsequent course of advancement.

## SUMMARIO IN INTERLINGUA

*Diabete In Le Industria*

1. Diabete esseva presente in 266 ex circa 20.000 obreros de petroleria. Isto es un incidentia de 1,3 pro cento.

2. Le diagnose de diabete esseva plus frequente in le gruppos de etate plus avantiate.

3. Circa 80 pro cento del casos esseva asymptomatic al tempore de lor detection.

4. Un historia familial de diabete esseva obtenite in solmente 27,9 pro cento del casos positive.

5. In 74 pro cento del casos, therapia a insulina esseva requirite pro effectuar un control del diabete.

6. In 55,3 pro cento del casos le control effectuate esseva considerate como "excellente" o "bon," durante que in 37,5 pro cento illo esseva solmente "satis bon" o "non satisfactori."

7. Grados clinicamente significative de morbo cardiovascular-renal esseva presente in 29,8 pro cento del patientes (i.e. in 26,6 pro cento de illes recipiente insulina e in 39,0 pro cento de illes non recipiente insulina).

8. Obesitate—amontante a 15 pro cento o plus secundo le standards del Metropolitan Life Insurance Company—esseva presente in 31,1 pro cento del casos (i.e. in 33,0 pro cento del patientes recipiente insulina e in 25,6 pro cento del patientes non recipiente insulina).

9. Un grande varietate de typos de labor esseva executate satisfacentemente per iste obreros.

10. Restrictiones in le genere de labor a executar esseva applicate a solmente dece obreros (i.e. 3,2 pro cento del gruppo diabetic total). Omnes pertineva al gruppo sub tractamento con insulina. Iste restrictiones, a generalmente parlar, prohibiva le conduction de vehiculos del compania e le manipulation de machinas pesante o a motion rapide.

11. Restrictiones a causa de concomitante morbo cardiovascular-renal esseva plus commun. Illos afficeva 23 obreros (i.e. 7,3 pro cento del gruppo total). Iste restrictiones esseva identic con illos usate in casos de morbo cardiovascular-renal sin complication per diabete.

12. Le individuos responsabile pro le curatela de juvene diabeticos deberea recommendar a illes le preparation pro typos de labor in que le manipulation de pesante machinas, le conduction de vehiculos, etc., non es importante al initio del empleo o durante le curso subsequente de avantiamento.

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# Recent Statistics on Diabetes

Rather surprisingly, the mortality from diabetes in the United States in the first nine months of 1958 was somewhat lower than in the corresponding period of 1957, according to provisional data for the two years based upon the 10 per cent sample of death certificates.* Up through May the rate was persistently higher than in the corresponding period of 1957, primarily reflecting the widespread prevalence of respiratory disease, an aftermath of the "Asian influenza" outbreak of late 1957. The cumulative increase to the end of May was approximately 5 per cent. Consequently, the decline recorded for the first nine months was due to the extraordinarily low rates from June through September which more than wiped out the increase recorded up to May. Figures for the urban industrial population, represented by the Industrial policyholders of the Metropolitan Life Insurance Company, showed an increase in the first nine months of 1958 as compared with 1957. This was in accord with the experience in the states and cities from which preliminary reports are routinely obtained (table 1).

It may be noted that the mortality from all causes of death in the United States for the first nine months of 1958 was slightly higher than in the corresponding period of 1957. It is likely, however, that the figure for the whole year will be about the same as for 1957, since the mortality record for the last quarter of 1957, when "Asian influenza" was at its peak, was relatively poor as compared with the last quarter of 1958.

The diabetes death rate for the two large Canadian cities, Toronto and Montreal, both showed a distinct increase in the first nine months of 1958 over the corresponding period of 1957. In both cities the increase was approximately 20 per cent.

Data for England and Wales for the first nine months of 1958 are available only for London Administrative County. For that period the death rate from diabetes

TABLE 1

Recent data on diabetes mortality
Deaths and death rates—January-September 1958 and 1957

| Area | Death rates per 100,000 | | Number of deaths | |
|---|---|---|---|---|
| | 1958 | 1957 | 1958 | 1957 |
| United States (10 per cent sample) | 15.6 | 15.9 | 2,010 | 2,019 |
| Metropolitan Life Insurance Company Industrial Policyholders | 15.7 | 14.8 | 1,995 | 1,953 |
| New York State | 18.6 | 17.7 | 2,324 | 2,174 |
| New York City | 20.5 | 17.7 | 1,231 | 1,073 |
| Maryland | 17.1 | 16.7 | 382 | 363 |
| Baltimore, resident | 24.9 | 25.1 | 183 | 184 |
| Boston | 15.9 | 15.1 | 98 | 94 |
| Philadelphia | 20.6 | 19.2 | 338 | 315 |
| Toronto | 20.9 | 17.1 | 103 | 84 |
| Montreal, resident | 16.5 | 13.9 | 142 | 118 |
| London (Administrative County) | 8.1 | 7.6 | 197 | 186 |
| | January-March | | January-March | |
| England and Wales | | | | |
| Total | 8.8 | 7.2 | 989 | 808 |
| Males | 6.0 | 5.0 | 324 | 268 |
| Females | 11.4 | 9.3 | 665 | 540 |

Note: Rates for the states and cities are based upon local estimates of population. United States data based upon the returns from a 10 per cent sample of death certificates received in vital statistics offices, as published in "Current Mortality Analysis," a monthly report of the National Office of Vital Statistics of the U. S. Public Health Service.

was moderately higher than in the same period of 1957. For the country as a whole the data for 1958, limited to the first quarter, showed a marked increase in diabetes mortality—of the order of 20 per cent—over the first quarter of 1957. The proportionate rise was about the same for males and females. For England also the major factor appears to be the high prevalence of respiratory disorders in the country in the first quarter of 1958.

The provisional regional data on mortality from diabetes in the United States for the first nine months of 1958, based upon the 10 per cent sample of death certificates, showed a reduction in the rate as compared with 1957 in all areas except the East North Central and East South Central regions (table 2). The changes tend to be relatively moderate. It is likely that final figures for the year will show more distinct changes from the pattern shown in the table because of the larger number of deaths involved and because of the impact of "Asian influenza" in the last quarter of 1957.

*Information received just before going to press shows that for the year 1958, the death rate from diabetes, based upon provisional figures, for the United States was 15.3 per 100,000 compared with 16.4 in 1957.

Submitted by the Committee on Statistics, Herbert H. Marks, Chairman. The Committee welcomes suggestions or actual materials suitable for this section in future issues from Association members and other readers of the Journal.

TABLE 2

Number of deaths and death rates from diabetes in geographic division: United States reporting area for the 10 per cent sample; January-September 1958, 1957 and 1956

| Geographic division | Death rates per 100,000* | | | Number of deaths* | | |
|---|---|---|---|---|---|---|
| | 1958 | 1957 | 1956 | 1958 | 1957 | 1956 |
| U.S. reporting area | 15.6 | 15.9 | 15.9 | 2,010 | 2,019 | 1,973 |
| New England | 20.3 | 21.1 | 19.3 | 139 | 155 | 137 |
| Middle Atlantic | 19.5 | 20.3 | 21.9 | 483 | 498 | 530 |
| East North Central | 19.1 | 18.0 | 19.8 | 508 | 470 | 570 |
| West North Central | 14.7 | 16.7 | 15.5 | 169 | 189 | 175 |
| South Atlantic | 13.3 | 13.5 | 11.5 | 251 | 249 | 207 |
| East South Central | 13.1 | 10.3 | 12.5 | 117 | 91 | 109 |
| West South Central | 12.6 | 12.7 | 9.6 | 156 | 155 | 114 |
| Mountain | 8.7 | 11.2 | 12.2 | 42 | 53 | 57 |
| Pacific | 10.3 | 11.6 | 10.0 | 145 | 159 | 134 |

*Excludes armed forces overseas.

Note: These data from the 10 per cent sample are subject to sampling error. The number of deaths, as given, does not cover the entire United States for each month but is limited by the completeness of the reporting area. The size of the reporting area is indicated by the footnote on page seven of each monthly issue of the "Current Mortality Analysis."

Source: Data furnished by National Office of Vital Statistics of the U.S. Public Health Service.

TABLE 3

Deaths and death rates from diabetes by age—United States, 1957 and 1956

| Age period (years) | Death rates per 100,000 | | Number of deaths | |
|---|---|---|---|---|
| | 1957* | 1956 | 1957* | 1956 |
| Total | 16.4 | 15.7 | 27,930 | 26,184 |
| Under 1 | 0.5 | 0.5 | 20 | 19 |
| 1-14 | 0.5 | 0.3 | 220 | 122 |
| 15-24 | 1.2 | 1.0 | 260 | 215 |
| 25-34 | 2.0 | 2.4 | 470 | 561 |
| 35-44 | 4.8 | 3.8 | 1,110 | 884 |
| 45-54 | 10.9 | 10.9 | 2,150 | 2,110 |
| 55-64 | 37.8 | 37.6 | 5,650 | 5,549 |
| 65-74 | 105.1 | 96.5 | 10,230 | 9,241 |
| 75-84 | 155.8 | 153.7 | 6,510 | 6,177 |
| 85 + | 157.3 | 160.4 | 1,310 | 1,296 |
| Not stated | — | — | — | 10 |

*Estimated from 10 per cent sample. Excludes armed forces overseas.

Source: National Office of Vital Statistics. For 1957, Monthly Vital Statistics Report, annual summary. For 1956, Vital Statistics of the U.S., Volumes I & II.

Provisional data by age groups on mortality from diabetes in the United States in 1957, now available, are shown in comparison with the corresponding final figures for 1956 (table 3). In interpreting the data it should be realized that the actual numbers in the 1957 sample are one tenth of the numbers shown in the table and certain of them are subject to considerable sampling error. Nevertheless, it is evident that the impact of the influenza outbreak on the death rate from diabetes was greatest at ages sixty-five and over.

TABLE 4

Crude and age-adjusted* death rates from diabetes—United States, 1949-1956 according to Sixth Revision

| Year | Death rates per 100,000† | |
|---|---|---|
| | Crude | Age-adjusted |
| 1956 | 15.7 | 13.3 |
| 1955 | 15.5 | 13.2 |
| 1954 | 15.6 | 13.3 |
| 1953 | 16.3 | 14.0 |
| 1952 | 16.4 | 14.2 |
| 1951 | 16.3 | 14.2 |
| 1950 | 16.2 | 14.3 |
| 1949 | 16.9 | 15.0 |

*Standardized on the basis of the total population of the United States in 1940.

†Excludes armed forces overseas.

Source: National Office of Vital Statistics of the United States Public Health Service, Special Reports-National Summaries.

Crude and age-adjusted death rates from diabetes for the United States from 1949 to 1956, the period in which the *Sixth Revision of the International List of Causes of Death* has been in use, are given in table 4. The trend of the age-adjusted rates has been almost continuously downward. The 1956 rate was 11 per cent less than that of 1949, as compared with a decline of only 7 per cent in the crude rate. This gives a measure of the influence of the changing age distribution of the country's population on the diabetes death rate over this period.

The age-adjusted death rates for diabetes by race and sex for 1956, along with comparison of the rates of change from the 1949 figures, are given in table 5. It is noteworthy first that the death rates for nonwhites are higher than those for whites—nearly two thirds higher in the case of females and one eighth higher in the case of males. Among white persons the death rate from diabetes among females was more than one fourth higher than among the males, but among nonwhites the rate among females was nearly double that among males. Changes in the rates since 1949 showed marked variation: Among white persons the rates have declined nearly one fifth in the case of females, whereas among nonwhites increases have been recorded for each sex—over one eighth in the case of males.

The trend of diabetes mortality in England and Wales also has been downward in recent years as shown in table 6. The rate in 1957 was one sixth lower than that in 1950. Unlike the situation in this country the rate of decline in England and Wales has been approximately the same in both sexes.

Detailed statistics of mortality from diabetes in the United States by color, sex and age for the year 1956 are given in table 7. The division by quinquennial age

TABLE 5

Age-adjusted death rates from diabetes—1956 and per cent change since 1949 by race and sex. United States

| | Total | Death rates per 100,000 White | Nonwhite |
|---|---|---|---|
| Persons | 13.3 | 12.8 | 18.1 |
| Males | 11.2 | 11.0 | 12.4 |
| Females | 15.1 | 14.2 | 23.4 |
| | | Per cent change since 1949 | |
| Persons | —11.3 | —12.9 | + 6.5 |
| Males | — 4.3 | — 6.0 | +12.7 |
| Females | —16.4 | —19.3 | + 1.7 |

Source: National Office of Vital Statistics, National Summaries, Vital Statistics-Special Reports, vol. 48, no. 8, Aug. 15, 1958.

TABLE 6

Number of deaths and death rates from diabetes by sex—England and Wales, 1950-1957

| Year | Death rate per 100,000 Total | Males | Females | Deaths Total | Males | Females |
|---|---|---|---|---|---|---|
| 1957 | 7.0 | 4.7 | 9.1 | 3,137 | 1,013 | 2,124 |
| 1956 | 7.3 | 5.1 | 9.2 | 3,242 | 1,108 | 2,134 |
| 1955 | 7.4 | 5.1 | 9.6 | 3,291 | 1,084 | 2,207 |
| 1954 | 6.8 | 4.9 | 8.6 | 3,028 | 1,048 | 1,980 |
| 1953 | 7.2 | 5.0 | 9.3 | 3,194 | 1,066 | 2,128 |
| 1952 | 7.6 | 5.2 | 9.8 | 3,338 | 1,091 | 2,247 |
| 1951 | 8.5 | 5.8 | 10.9 | 3,703 | 1,219 | 2,484 |
| 1950 | 8.4 | 5.8 | 10.9 | 3,684 | 1,221 | 2,463 |

Source: The Registrar General's Statistical Review of England and Wales for the year 1957, Part I.

TABLE 7

Death rates from diabetes mellitus by race, sex and age—United States, 1956

| Age period (Years) | Death rates per 100,000* Total | White Male | White Female | Nonwhite Male | Nonwhite Female |
|---|---|---|---|---|---|
| All ages | 15.7 | 12.8 | 18.6 | 10.0 | 19.2 |
| Under 1 | 0.5 | 0.4 | 0.7 | 0.4 | — |
| 1- 4 | 0.2 | 0.2 | 0.2 | — | 0.2 |
| 5- 9 | 0.2 | 0.1 | 0.2 | 0.2 | 0.3 |
| 10-14 | 0.4 | 0.4 | 0.5 | 0.1 | 0.6 |
| 15-19 | 0.7 | 0.4 | 0.9 | 1.1 | 1.7 |
| 20-24 | 1.3 | 1.1 | 1.2 | 1.7 | 2.9 |
| 25-29 | 2.0 | 1.9 | 1.7 | 3.3 | 3.3 |
| 30-34 | 2.7 | 3.3 | 1.9 | 3.4 | 4.1 |
| 35-39 | 3.3 | 3.5 | 2.5 | 6.8 | 6.2 |
| 40-44 | 4.4 | 4.5 | 3.0 | 6.1 | 13.0 |
| 45-49 | 7.3 | 6.2 | 5.6 | 14.1 | 25.6 |
| 50-54 | 15.1 | 13.0 | 13.3 | 15.6 | 51.4 |
| 55-59 | 27.8 | 21.6 | 27.4 | 41.7 | 82.1 |
| 60-64 | 49.1 | 38.9 | 53.8 | 49.4 | 108.9 |
| 65-69 | 83.0 | 62.6 | 98.2 | 77.3 | 139.6 |
| 70-74 | 114.2 | 90.8 | 134.2 | 82.0 | 143.8 |
| 75-79 | 143.5 | 123.1 | 166.0 | 76.7 | 116.8 |
| 80-84 | 173.6 | 161.5 | 192.3 | 78.6 | 113.0 |
| 85 + | 160.4 | 156.9 | 179.6 | 44.1 | 84.1 |

*Excludes armed forces overseas.

Note: The death rates which are underscored are based on less than twenty-five deaths.

Source: United States Department of Health, Education and Welfare, Public Health Service, National Office of Vital Statistics Special Reports-National Summaries, volume 48, number 8, Aug. 15, 1958.

groups yields numbers of deaths in many groups that are relatively small, especially among nonwhites (under twenty-five deaths in most sex-age groups among whites under age twenty, and in nonwhites under age thirty-five; in the latter under ten deaths in all but one sex-age division under twenty years). Consequently, caution is needed in making comparisons involving these groups. In general in all groups there was a steady upward increase in the death rates with age to a peak at ages eighty to eighty-four in the aggregate and in white persons of both sexes. In nonwhites the peak occurred at ages seventy to seventy-four. Among white persons the rates for males exceeded those of females in each five-year age group from twenty-five to forty-nine years. The differences were especially large in the thirties and early forties. White females had the higher rates in all age groups over fifty, the margin being proportionately greatest at ages sixty-five to seventy-four. Among nonwhites the rates for females were consistently higher than those for males in all age groups over forty. The margin was relatively greatest at ages fifty to fifty-four when the rate for females was more than three times as high as for males. At ages fifty-five to sixty-four the rates were double or more those for males.

The wide variations between countries in the mortality ascribed to diabetes at all ages and in middle life are brought out in table 8 which covers the three-year period, 1953-1955. The data for the United States and several European countries are presented by sex and age. The countries are listed in order of the rank of age-adjusted death rates for males at ages forty to sixty-four. In interpreting the data the reader is cautioned that practices in certifying diabetes and procedures in classifying death certificates in which diabetes is reported vary from country to country.

Among both males and females the highest rate at all ages in the countries listed was reported for Belgium, but at ages forty to sixty-four the age-adjusted rate in the United States was the highest. Among males at all ages the lowest rate was recorded for Finland, but at ages forty to sixty-four for England and Wales—less than one fourth the rate for the United States. Among females the rate for Denmark was lowest at all ages, but at forty to sixty-four was lowest for Norway and almost equally low for England and Wales, and Austria

TABLE 8

Mortality from diabetes mellitus at all ages and ages forty to sixty-four in the United States and certain European countries, 1953-1955 (ranked in order of age-adjusted rates for males at ages forty to sixty-four years)

| Country | Death rates per 100,000 | | | | | | | | Per cent diabetes of all deaths | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | All Ages | 40-64* | 40-64 | 40-44 | 45-49 | 50-54 | 55-59 | 60-64 | All Ages | 40-64 | 40-44 | 45-49 | 50-54 | 55-59 | 60-64 |
| **MALES** | | | | | | | | | | | | | | | |
| United States | 12.8 | 15.3 | 15.3 | 5.1 | 7.3 | 13.1 | 22.5 | 39.1 | 1.2 | 1.2 | 1.1 | 0.9 | 1.0 | 1.2 | 1.4 |
| Switzerland† | 10.3 | 11.8 | 11.4 | 2.8 | 3.6 | 10.0 | 17.6 | 34.6 | 1.0 | 1.1 | 0.8 | 0.6 | 1.1 | 1.2 | 1.5 |
| Belgium‡ | 14.7 | 10.2 | 10.3 | 1.7 | 2.2 | 5.7 | 18.7 | 32.8 | 1.1 | 0.9 | 0.5 | 0.3 | 0.5 | 1.1 | 1.3 |
| Italy§ | 8.5 | 9.3 | 8.6 | 1.4 | 3.0 | 6.3 | 14.7 | 29.8 | 0.9 | 0.9 | 0.4 | 0.5 | 0.7 | 1.0 | 1.4 |
| France† | 8.0 | 8.6 | 8.3 | 2.5 | 4.6 | 6.9 | 12.3 | 22.7 | 0.6 | 0.7 | 0.5 | 0.6 | 0.6 | 0.7 | 0.9 |
| Germany—Fed. Rep. | 7.6 | 7.2 | 7.1 | 1.7 | 3.4 | 5.7 | 9.7 | 21.5 | 0.7 | 0.7 | 0.5 | 0.6 | 0.6 | 0.6 | 0.9 |
| Sweden† | 8.4 | 6.3 | 6.2 | 3.7 | 3.7 | 5.0 | 8.9 | 13.0 | 0.8 | 0.8 | 1.4 | 0.8 | 0.7 | 0.8 | 0.7 |
| Denmark | 4.5 | 5.8 | 5.8 | 3.2 | 2.8 | 4.7 | 6.8 | 15.2 | 0.5 | 0.7 | 1.2 | 0.6 | 0.6 | 0.6 | 0.8 |
| Scotland | 5.4 | 5.3 | 5.2 | 3.4 | 2.3 | 4.0 | 8.1 | 11.7 | 0.4 | 0.4 | 0.9 | 0.3 | 0.3 | 0.4 | 0.4 |
| Netherlands | 7.3 | 5.2 | 5.0 | 1.6 | 1.7 | 2.7 | 8.1 | 16.5 | 0.9 | 0.7 | 0.6 | 0.4 | 0.4 | 0.7 | 0.9 |
| Finland | 3.9 | 5.1 | 4.8 | 1.7 | 3.9 | 5.4 | 5.2 | 11.9 | 0.4 | 0.3 | 0.3 | 0.5 | 0.4 | 0.2 | 0.4 |
| Austria | 5.2 | 4.9 | 4.9 | 1.8 | 2.6 | 2.9 | 7.5 | 13.0 | 0.4 | 0.4 | 0.5 | 0.4 | 0.3 | 0.4 | 0.5 |
| Norway† | 5.6 | 4.9 | 4.8 | 3.9 | 3.1 | 3.3 | 5.4 | 10.6 | 0.6 | 0.7 | 1.4 | 0.7 | 0.5 | 0.5 | 0.6 |
| England and Wales | 5.0 | 3.7 | 3.7 | 1.2 | 1.8 | 3.1 | 5.2 | 9.9 | 0.4 | 0.3 | 0.4 | 0.3 | 0.3 | 0.3 | 0.4 |
| **FEMALES** | | | | | | | | | | | | | | | |
| United States | 19.0 | 21.9 | 22.0 | 4.3 | 8.2 | 17.2 | 34.3 | 64.0 | 2.4 | 3.0 | 1.4 | 1.8 | 2.5 | 3.4 | 4.1 |
| Switzerland† | 17.7 | 13.8 | 14.1 | 1.6 | 4.1 | 9.5 | 21.5 | 45.6 | 1.9 | 2.2 | 0.7 | 1.1 | 1.6 | 2.3 | 3.1 |
| Belgium‡ | 32.9 | 20.0 | 21.6 | 2.2 | 5.1 | 14.1 | 29.2 | 69.3 | 3.0 | 3.2 | 0.9 | 1.3 | 2.5 | 3.2 | 4.7 |
| Italy§ | 12.6 | 12.0 | 12.0 | 1.3 | 3.1 | 7.4 | 19.2 | 40.9 | 1.5 | 2.0 | 0.5 | 0.9 | 1.4 | 2.3 | 2.9 |
| France† | 14.2 | 10.2 | 11.1 | 1.9 | 3.0 | 8.0 | 16.2 | 31.1 | 1.2 | 1.5 | 0.6 | 0.7 | 1.2 | 1.7 | 2.2 |
| Germany—Fed. Rep. | 12.8 | 9.9 | 10.0 | 1.4 | 3.0 | 6.7 | 16.3 | 31.2 | 1.3 | 1.5 | 0.5 | 0.8 | 1.2 | 1.8 | 2.1 |
| Sweden† | 13.7 | 8.6 | 8.8 | 1.7 | 3.0 | 5.9 | 11.5 | 28.2 | 1.5 | 1.5 | 0.8 | 0.9 | 1.1 | 1.4 | 2.0 |
| Denmark | 6.3 | 6.5 | 6.6 | 1.9 | 2.2 | 3.5 | 9.8 | 20.8 | 0.7 | 1.1 | 0.8 | 0.6 | 0.6 | 1.1 | 1.5 |
| Scotland | 11.8 | 10.4 | 10.8 | 1.6 | 3.7 | 5.6 | 17.4 | 33.5 | 1.1 | 1.4 | 0.5 | 0.8 | 0.8 | 1.6 | 1.9 |
| Netherlands | 16.5 | 9.1 | 9.2 | 1.0 | 2.1 | 4.9 | 14.6 | 32.5 | 2.3 | 1.7 | 0.5 | 0.7 | 1.0 | 1.9 | 2.5 |
| Finland | 7.6 | 6.3 | 6.0 | 1.1 | 1.4 | 5.9 | 7.9 | 20.8 | 0.9 | 0.9 | 0.4 | 0.3 | 0.9 | 0.8 | 1.2 |
| Austria | 9.2 | 5.9 | 6.2 | 0.8 | 2.2 | 3.9 | 9.3 | 18.9 | 0.8 | 0.9 | 0.3 | 0.5 | 0.6 | 1.0 | 1.2 |
| Norway† | 7.7 | 5.7 | 5.7 | 3.1 | 2.0 | 3.8 | 8.2 | 15.2 | 0.9 | 1.1 | 1.7 | 0.7 | 0.8 | 1.1 | 1.3 |
| England and Wales | 9.2 | 5.8 | 6.3 | 1.5 | 2.1 | 3.8 | 9.0 | 17.6 | 0.9 | 1.0 | 0.6 | 0.6 | 0.7 | 1.0 | 1.3 |

*Standardized on the basis of the total population of the United States as of April 1, 1950.
†1952-1954 ‡1954-1955 §1954
Source: United States—Basic data from reports of National Office of Vital Statistics.
Europe—Epidemiological and Vital Statistics Report, Vol. 10, World Health Organization, Geneva, 1957.

—a little more than one fourth the rate for the United States. Among both males and females the rates for the United States were considerably higher in each quinquennial age group from forty to sixty-four, except at sixty to sixty-four among females where the rate for Belgium was moderately the higher. In general the rank of the countries in the order of size of the diabetes death rate showed only moderate shifts from one age group to the next in each sex, the major exceptions being for age groups under fifty in countries with relatively small populations. At the older ages there were some significant exceptions, e.g., Dutch and English males at ages sixty to sixty-four and Dutch and Scottish females at ages fifty-five to sixty-four. Also, whereas the rates for Swiss males between ages forty and sixty-four tended to be higher than for the Belgian males, the reverse was true in the case of females.

Within the age range forty to sixty-four years, the rise in mortality rates with age generally was sharper in females than in males. In fact, rates for males in most countries were higher than those for females at ages forty to forty-four and in the majority of the countries listed at ages forty-five to forty-nine also, but after fifty the rates for females were the higher with but few exceptions.

In the age range forty to sixty-four years, diabetes accounts for an appreciably greater percentage of the total mortality among females than among males. This primarily reflects two factors—the higher death rates from diabetes among women in the older age groups combined with the lower death rate from all causes. Thus, while in most countries diabetes among males accounts for only a fraction of 1 per cent of all deaths in every age group, it exceeds 1 per cent in females in a large number of countries and in virtually all countries for the age groups fifty-five to sixty-four years.

The maximum proportion—more than 4 per cent—was recorded for females in the United States and Belgium at ages sixty to sixty-four.

The *Seventh Revision of the International List of Causes of Death* became available for use at the beginning of 1958. The National Office of Vital Statistics and state and local registration offices then started to classify death certificates by this revision. While the changes incorporated in the list for the Seventh Revision are limited in extent, the combination of these and certain procedural changes have affected the comparability of certain categories to an appreciable extent. Provisional figures showing the combined effects of these changes made in classification and procedures were tabulated by the National Office of Vital Statistics on the basis of the 10 per cent sample of death certificates for January to June 1958 which were classified by both revisions. The results were expressed in terms of the ratio of Seventh to Sixth Revision assignments. Selected categories of causes from the tabulation which have some interest in relation to diabetes are given in table 9. For diabetes the comparability ratio was 1.02; in other words deaths ascribed to diabetes by the Seventh Revision were 2 per cent higher than by the Sixth Revision.

Rather appreciable changes appear in assignments within the broad category of the "Major cardiovascular-renal diseases" although the comparability ratio for the more important aggregates within this particular group indicates little change. The major item here is modification of the practice in classifying certain death certificates involving "Arteriosclerotic heart disease" so described. If myocardial disease or hypertensive disease is mentioned with arteriosclerotic heart disease in the Cause of Death Statement, the death is classified under the specific myocardial or hypertensive category applicable, whereas under the Sixth Revision all such cases would have been classified as arteriosclerotic heart disease. Hence the comparatively high comparability ratios for "Other diseases of the heart," "Hypertensive heart disease," and "Other diseases of the circulatory system."

The major procedural change is with respect to pneumonia. A study by the National Office of Vital Statistics indicated that lobar pneumonia, when certified in the presence of degenerative diseases, most frequently was intended to mean a terminal pneumonia. For example, unless there are clear indications to the contrary, deaths involving lobar pneumonia in persons with coronary disease are assigned to coronary disease under the Seventh Revision, whereas previously they were classified under pneumonia. This is one of the factors lowering the comparability ratio for pneumonia and raising the ratios for diabetes and certain cardiovascular disorders.

The comparability ratios are likely to change still further beginning with 1959, partly because of changes made in procedure as a result of a critical review of the use of the Seventh Revision in 1958, and partly because of a change in the "underlying cause" concept. The latter involves the extension of the concept to conditions which may be considered "predisposing," as well as those which may be considered directly causative.

TABLE 9

Provisional comparability ratios for certain causes—United States, January-June 1958

| Cause of death (Seventh Revision of the International Lists, 1955) | Ratio* |
|---|---|
| Diabetes mellitus | 1.02 |
| Malignant neoplasms | 0.99 |
| Major cardiovascular-renal diseases | 1.01 |
| Diseases of cardiovascular system | 1.01 |
| Vascular lesions affecting central nervous system | 1.00 |
| Rheumatic fever | 0.94 |
| Diseases of heart | 1.00 |
| Chronic rheumatic heart disease | 0.99 |
| Arteriosclerotic heart disease, including coronary disease | 0.98 |
| Nonrheumatic chronic endocarditis and other myocardial degeneration | 0.98 |
| Other diseases of heart | 1.13 |
| Hypertensive heart disease | 1.11 |
| General arteriosclerosis | 1.01 |
| Other diseases of circulatory system | 1.17 |
| Chronic and unspecified nephritis and other renal sclerosis | 1.03 |
| Influenza and pneumonia, except pneumonia of newborn | 0.95 |
| Influenza | 1.05 |
| Pneumonia, except pneumonia of newborn | 0.93 |

*Ratio of deaths in the 10 per cent sample for January-June 1958 classified by the Seventh Revision to deaths classified by the Sixth Revision.

Source: Monthly Vital Statistics Report. National Office of Vital Statistics, Volume 7, Number 9, Nov. 17, 1958.

# EDITORIAL

## ACTIONS OF PHENETHYLBIGUANIDE AND RELATED COMPOUNDS

Recognition of the hypoglycemic activity of certain sulfonamide derivatives has stimulated a great revival of interest in the possibility of effective oral treatment for diabetics. In the case of the sulfonylureas it has become increasingly clear that several mechanisms may be involved in their action, but that these are in turn dependent upon the presence of a considerable amount of functioning pancreatic islet tissue. Several years ago a new group of compounds, chemically unrelated to the sulfonylureas, were found by Ungar and co-workers[1] to have marked hypoglycemic activity in several animal species and also in alloxan diabetic animals. The active chemical grouping is a condensed diguanidine, or biguanide, with which several simple side chains have been combined to form compounds such as phenethylbiguanide (now familiar as PEBG or DBI*), amylbiguanide (ABG), or isoamylbiguanide. These drugs bear a considerable chemical resemblance to the diguanidines, most notable of which is Synthalin A, or decamethylenediguanidine, which was used for oral diabetic therapy for a brief time about thirty years ago, but was later abandoned for a number of reasons including observations of hepatic and renal toxicity in experimental animals. Although the biguanides produce metabolic effects which are similar to those of Synthalin, they are not as potent and do not seem to share its severe histotoxicity, which may stem in part from the 10 carbon saturated hydrocarbon chain in the Synthalin molecule. Several investigators have administered biguanides chronically in low doses to animals and have observed no histologic changes, and thus far no alterations in liver or renal function have been detected in diabetic humans receiving these agents for relatively long periods of time. Creutzfeldt and Moench,[2] however, have observed that phenethylbiguanide, administered to guinea pigs in somewhat higher doses than were used in other toxicity studies, produces degranulation and vacuolization of alpha cells of the pancreatic islets and degenerative changes in the liver and kidney tubules which clearly resemble the effects of Synthalin A or B, but are of relatively mild degree. These latter observations are not surprising when considered in the light of certain biochemical effects which are common to both groups of guanidine derivatives.

Clinical trials with the biguanides by a number of investigators have revealed several facets of their action which, when fully understood, will undoubtedly add to our fundamental knowledge of carbohydrate metabolism, regardless of the ultimate usefulness of these drugs in long-term diabetic therapy. First of all is the observation that biguanides exert a distinct blood-sugar-lowering effect even in diabetics who can be regarded as having little or no pancreatic islet function, and this is in keeping with the hypoglycemic effect of these drugs in alloxanized and depancreatized animals, and with their apparent lack of effect upon insulin secretion or degradation. A minimal amount of endogenous or exogenous insulin is necessary, however, or acidosis will develop. The second observation is that these agents augment the hypoglycemic effect of a given dose of insulin in the diabetic. It should be clearly understood that while the drugs enhance the hypoglycemic effectiveness of insulin, they have not been demonstrated to simulate or enhance any of its other metabolic effects, and in fact, in sufficient dosage may actually antagonize or prevent some of these such as glycogen deposition and fat and protein synthesis. The reason for this disparity between an insulin-like effect upon glucose uptake and an insulin-antagonistic action on certain other cellular processes can be readily understood when the biochemical effects of insulin and the guanidine derivatives are compared.

Experiments with guinea pigs summarized elsewhere[3] have demonstrated that PEBG increases the peripheral uptake of glucose. This effect can be seen in eviscerated preparations and also is readily demonstrable in vitro with the isolated rat hemidiaphragm. In contrast to the effect with insulin, muscle glycogen deposition does not occur, and in fact, glycogen stores are diminished. In adipose tissue in vitro, oxidation of glucose to $CO_2$ and fat synthesis are both depressed by PEBG, in contrast to the marked stimulatory effect of insulin on these processes.[4] In addition to this effect on peripheral tissues, biguanides appear to decrease the hepatic production of new glucose as determined by inferior vena cava and hepatic venous glucose differences in guinea pigs. Further evidence that gluconeogenesis may be inhibited are the observations that urea production is decreased and that alanine fails to influence the very low liver glycogen content or the blood glucose level in treated animals.

*DBI is an abbreviation applied in the early investigations of this compound, which was derived from "*dia*betic number *1*."

Decreased urea production cannot result from inhibition of its formation from arginine in the liver's urea cycle, since the enzyme arginase is not inhibited by PEBG,[5] and therefore, it seems reasonable to assume that it is the deamination of amino acids and their conversion to glucose which is decreased.

Since the peripheral uptake of glucose is increased while its conversion to glycogen and fat is decreased, the question arises as to the fate of this sugar. Most of it appears to be converted to lactic acid which accumulates in the incubation medium in in vitro experiments, or in the blood and to some extent the urine in vivo. These observations indicate that anaerobic glycolysis of glucose is accelerated in the tissues, but it is unlikely that this is the result of a direct stimulatory effect of biguanides upon any of the enzymes of the Embden-Meyerhof pathway or upon the processes involved in glucose entry into the cell. The principal enzymatic site of action of these agents, as well as other guanidine derivatives such as Synthalin, appears to be upon certain enzyme systems of the mitochondria which are concerned with Krebs' Cycle oxidations and the generation of high energy phosphates such as ATP.[3,4,6] The consumption of oxygen and the oxidation of all the intermediates of the tricarboxylic acid cycle have been shown to be depressed in all tissues tested as well as in isolated mitochondria from liver or kidney when incubated with PEBG or other guanidine derivatives. If sufficient amounts of these compounds are added, respiration can be arrested almost completely. The exact site of this inhibition within the mitochondria in vivo is not certain and may actually involve several enzyme complexes concerned with electron transport or oxidative phosphorylation. Cytochrome oxidase activity was found to be significantly inhibited in vitro by biguanides and Synthalin A in our studies.[3] However, after extensive study of the effects of guanidine and Synthalin on mitochondrial respiration and oxidative phosphorylation, Hollunger[6] concluded that the enzymatic process concerned with the coupling of phosphorylation to electron transport was affected by guanidine rather than any of the enzymes concerned with electron transport per se. His results do not necessarily contradict our own, inasmuch as the effect of Synthalin upon cytochrome oxidase was not studied in his experiments. Since the mechanism of oxidative phosphorylation has not been completely elucidated, definitive proof of inhibition by guanidines or biguanides at this level is necessarily lacking, whereas demonstration of inhibition of electron transport in disrupted mitochondrial particles which do not exhibit coupling is nonetheless possible. Both of these mechanisms apply to the same chain of linked reactions and either would produce the same end result of inhibition of the oxidations of the tricarboxylic acid cycle as well as many other oxidative processes of the mitochondria including the formation of high energy phosphates. Such an action would be quite comparable to the effect of oxygen deprivation or anaerobiosis.

It has been known since the days of Pasteur that anaerobiosis stimulates the uptake and glycolysis of glucose by micro-organisms and many animal cells. Evidently the rate of entry of glucose into the cells is normally governed by oxidative activity through a mechanism which has not been elucidated. Under anaerobic conditions this inhibitory effect is abolished and glucose entry proceeds at a greatly accelerated rate. This regulatory mechanism seems to occur in both insulin sensitive and insulin insensitive cells, and may be regarded as an insulin independent process since this so-called "Pasteur Effect" can be demonstrated in a tissue such as the rat hemidiaphragm in vitro without added insulin. Randle and Smith[7] have examined the effect of anaerobiosis and a number of oxidative poisons on the glucose uptake of the rat hemidiaphragm and their results show conclusively that this mechanism is present and that it can be demonstrated particularly well by the use of a bicarbonate buffering system. These authors emphasize the well-known fact that insulin can increase the glucose uptake without affecting the oxidative activity of the tissue. Thus it seems to be capable of overcoming the restraining influence on glucose entry, which aerobic metabolism imposes, without interfering with intracellular processes essential for the efficient utilization of nutrients in the production of energy and the synthesis of cellular materials. A wide variety of organic and inorganic substances can increase the glucose uptake of the rat hemidiaphragm by virtue of their ability to interfere with normal oxidation, but this additional glucose cannot be effectively used metabolically and the major portion is metabolized only to lactic acid. Thus the observation of an increased glucose uptake cannot be simply equated to an insulin-like action. Experiments with the rat hemidiaphragm emphasize that the biguanide effect upon glucose uptake depends upon inhibition of mitochondrial oxidations, inasmuch as no increase in glucose uptake occurs unless sufficient biguanide is added to inhibit oxygen consumption. If the hemidiaphragms are incubated under anaerobic conditions, PEBG does not cause any increase in glucose uptake. Thus far no chemical agents have been found, with the exception of insulin, which can act directly upon the process which normally regulates the aerobic uptake of glucose. Further elucida-

tion of this interesting mechanism should be very rewarding for it may then be possible to understand why the insulin requirement of the diabetic is lowered during active muscular work, as well as during biguanide therapy. In both these instances anaerobic glycolysis is increased and lactic acid accumulation occurs.

Turning to the effect of the biguanides upon the hepatic production of glucose, it is possible to extend the observations of respiratory inhibition to this organ and to relate these effects in experimental animals. Inhibition of the respiration of rat liver slices can be demonstrated both on addition of PEBG in vitro and after in vivo administration. The marked lowering of hepatic glycogen and the failure of glucose or a glycogenic amino acid such as alanine to prevent the decrease is attributable to this hypoxic action. Many of the enzymatic processes necessary for the deamination of amino acids and their conversion to pyruvate depend upon the operation of portions of the tricarboxylic acid cycle and the electron transport system. Moreover, the conversion of pyruvate to glucose is an energy requiring synthesis which utilizes ATP generated by mitochondrial oxidations. Gluconeogenesis is thus directly linked to the oxidative activity of the liver and could not continue in the absence of an adequate aerobic metabolism. The degree to which gluconeogenesis would be depressed in vivo would depend, theoretically, upon the extent to which oxidative metabolism was inhibited and this, of course, would depend upon the dose administered and the degree to which the drug accumulates in the liver of the particular species under consideration. Using $C^{14}$-labeled PEBG, Wick[8] has found significant accumulation of the drug in the liver of rats. A small number of observations with ordinary doses of PEBG in humans, however, suggests that inhibition of gluconeogenesis does not occur at all or may be so minimal as to escape detection. Thus far no changes have been found in hepatic oxygen consumption, urea production, glucagon responsiveness, or glucose output in normal or diabetic human subjects.[9,10]

It has not been conclusively demonstrated, on the other hand, that the hypoglycemia in diabetic humans is due to increased peripheral uptake of glucose. Elevations of blood lactate and pyruvate have been noted, and pyruvate tolerance is decreased after PEBG administration to humans.[10] Since the liver is normally able to dispose of large amounts of these substances it seems reasonable to suspect that hepatic metabolism may indeed be affected in vivo by these drugs. Although insulin also causes a slight rise in blood lactate, in contrast to the biguanides, it increases pyruvate utilization in diabetics, and lowers the blood content of citric acid. Elevations of blood lactate, pyruvate, phosphate and citrate have been reported to occur following the administration of a variety of inhibitors of aerobic metabolism including guanidines and Synthalin. These changes, therefore, originate from a mechanism quite distinct from that of insulin and should not be confused with any of its metabolic effects. The demonstration of some of these changes in human subjects lends support to the view that the biguanides affect aerobic metabolism to some extent. It seems likely that this may occur only to a very slight extent with the doses employed and further knowledge of the distribution of these compounds in humans is needed to determine which tissues may be principally affected.

Although experimental evidence is lacking that the hypoglycemic mechanism outlined in figure 1 is applicable to the treated human diabetic, the enzymatic

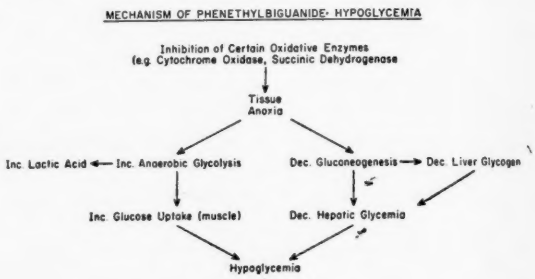

FIGURE 1

and animal studies provide a framework of information against which clinical observations may be partially interpreted, and further, they indicate the need for the most thoughtful and thorough evaluation of the potential usefulness and ultimate benefit to the diabetic patient of any new agents which may be found to lower the blood glucose concentration. In this process of evaluation the clinical trial is equally as important as the laboratory, so long as the over-all benefits to the patient are objectively assessed in as many ways as possible.

REFERENCES

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[4] Wick, A. N., Larson, E. R., and Serif, G. S.: A site of action of phenethylbiguanide—a hypoglycemic compound. J. Biol. Chem. 233:296, 1958.

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[9] Tranquada, R., Kleeman, C., and Brown, J.: The acute effects of DBI on human hepatic intermediary metabolism. Clin. Res. 7:110, 1959.

[10] Moorhouse, J. A., Fajans, S. S., and Conn, J. W.: The effect of phenethylbiguanide on pyruvate utilization in man. Clin. Res. 6:405, 1958.

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# ABSTRACTS

*Aktan, Hamdi S.; and Klotz, Arthur P.* (Dept. of Med., Univ. of Kansas Med. Center, Kansas City, Kan.; Med. Serv. & Radioisotope Lab., Vet. Admin. Hosp., Kansas City, Mo.): FAT ABSORPTION AND PANCREATIC FUNCTION IN DIABETES MELLITUS. Ann. Int. Med. *49*:820-28, October 1958.

A study was conducted in ten normal adults and ten diabetic patients of fat absorption and pancreatic exocrine function. The former was studied by an $I^{131}$-iodotriolein absorption test and the latter by intravenous secretin. Fat absorption was normal in uncomplicated diabetes. It was abnormal in two patients with diabetic diarrhea while having the diarrhea but normal in one during remission. The secretin test was abnormal only in one diabetic patient complicated by chronic pancreatitis. S.B.B.

*Assevero, Victor L.; and Moss, James M.* (Howard University and Georgetown University, Washington, D.C.): DIABETES MELLITUS IN INFANCY: CASE REPORT. J. Pediat. *53*:227-30, August 1958.

The patient discussed was a one-month-old infant whose mother was not diabetic, who failed to gain weight, and entered hospital in dehydration. Glycosuria on one occasion was dismissed as due to clysis. Low sodium and high potassium initiated the incorrect diagnosis of adrenal insufficiency. Eventual management with NPH insulin 2 units twice daily was undertaken with gain in weight from 5 lb. to 8 lb. in two and a half months. R.L.J.

*Aszkanazy, C. L., Jenkins, L., and Simpson, W. W.* (Dept. of Pathology, and Med., Univ. of British Columbia and Vancouver Gen. Hosp., Vancouver, B.C., Canada): ADRENAL CORTICAL CARCINOMA ASSOCIATED WITH HYPOGLYCAEMIA. Canad. M. A. J. 79:482-84, Sept. 15, 1958.

A case of chronic hypoglycemia with terminal fatal hypoglycemia is described in association with carcinoma of the adrenal cortex in a twenty-six-year-old woman. Classical clinical features of Cushing's syndrome were absent. The tumor showed no insulin on hormonal assay to explain the hypoglycemia. S.B.B.

*Barber, Donn J.* (Greeley, Colorado): THE NEUROLOGIC COMPLICATIONS IN DIABETIC CHILDREN. Rocky Mountain M. J. *54*:809-13, August 1957.

The author discusses the neurologic changes that occur in children with diabetes, classifying these changes into two general classifications, cerebral damage from hypoglycemia and neurologic disorders produced by prolonged hyperglycemia and ketonuria. The physiology of the cerebral damage from hypoglycemia is discussed and the importance of a liberal control in preventing hypoglycemic reactions is stressed. There is also a discussion of the peripheral, neurologic changes occurring from hyperglycemia and it is again stressed that these can be prevented by adequate control. R.C.C.

*Beck, John C.; McGarry, E. E.; Dyrenfurth, I.; and Venning, E. H.* (McGill Univ. Clin., Royal Victoria Hosp., Montreal, Quebec, Canada): THE METABOLIC EFFECTS OF HUMAN AND MONKEY GROWTH HORMONE IN MAN. Ann. Int. Med. *49*:1090-1105, November 1958.

Metabolic studies of the effects of primate (human and monkey) growth hormone administration to six human subjects are described. One was normal, three had dwarfism, one had been hypophysectomized and one had postmenopausal osteoporosis. Both types of preparation resulted in metabolic effects (retention of nitrogen, calcium, phosphorus, sodium and potassium) and increased aldosterone excretion. However, the human (and porcine), but not the monkey, preparation caused decreased carbohydrate tolerance as measured by the glucose tolerance test. The results are discussed with reference to possible differences in chemical configuration of growth hormone derived from various species. S.B.B.

*Berson, Solomon A.; and Yalow, Rosalyn S.* (Veterans Administration Hosp., New York 68, N. Y.): INSULIN ANTAGONISTS, INSULIN ANTIBODIES AND INSULIN RESISTANCE. Am. J. Med. *25*:155-59, August 1958.

This is a comprehensive review of insulin antagonists, insulin antibodies, and insulin resistance in which the authors attempt to unravel the confusion regarding the kinship of the various factors and the nature of the opposition of these factors to insulin. R.W.S.

*Bickford, A. F.; and Mottram, R. F.* (Dept. of the Regius Professor of Medicine, The Radcliffe Infirmary, Oxford, England): GLUCOSE METABOLISM DURING PERIODS OF INDUCED HYPOTHERMIA. J. Physiol. *143*:37-38, Sept. 23, 1958.

Studies on the metabolism of glucose in hypothermic rabbits have been made using the intravenous glucose tolerance test, arteriovenous blood glucose differences and the respiratory quo-

tient. Measurements made at normal body temperature were compared with those in hypothermia (rectal temperature 25-30° C.) in seventeen rabbits observed over three- to five-hour periods. In all rabbits tested the respiratory quotient was low. Following the intravenous injection of glucose (0.5 gm. glucose/kilogram body weight) the tested animals excreted on the average 25 per cent of that injected. In one rabbit, however, the amount of glucose in the urine exceeded the amount injected, indicating that the release of glucose into the blood can still occur during hypothermia. In fifteen of the seventeen hypothermic rabbits at least one out of the three tests for glucose metabolism indicated that glucose was leaving the extracellular space, or was being oxidized by the cells. G.A.W.

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*Bowyer, Freda; and Widdas, W. F.* (Dept. of Physiol., King's College, London, W.C. 2, England): THE ACTION OF INHIBITORS ON THE FACILITATED HEXOSE TRANSFER SYSTEM IN ERYTHROCYTES. J. Physiol. *141*:219-32, April 30, 1958.

The entry and exit rate constants for the transport of glucose across the human red cell membrane have been determined over a range of concentrations from 38 to 76 millimolar. Although the constant for entry exceeds that for exit, it has been shown that the major part of this difference is due to approximations in the equations used, and that to postulate an asymmetrical transfer system is unjustifiable. In fact a symmetrical system of transfer can also account for the different degrees of entry and exit observed in experiments with inhibitors such as urethane and polyphloretin phosphate. The inhibitory reaction of dinitrofluorobenzene with the red cell appears to be irreversible, and the rate of development of inhibition appears to be accelerated by glucose. The rate of glucose exit is affected more than that of entry. It is difficult to account for these results in terms of a noncompetitive inhibition, and the possibility of an "internal competitor" is discussed. G.A.W.

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*Country Practitioner.* DIABETES MELLITUS IN CHILDREN (Correspondence). Brit. M. J. *2*:688-89, Sept. 13, 1958.

The author reports the occurrence of diabetes in a child at the age of nine months. Reference is made to the rare occurrence of this disease in children under one year of age. C.A.R.

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*Diller, W. F.; and Kilpatrick, R.* (Dept. of Pharmacol. and Therapeutics, University of Sheffield, England): ADRENALINE IN HYPERTHYROIDISM AND INSULIN HYPOGLYCAEMIA. Brit. M. J. *2*:823-25, Oct. 4, 1958.

The authors measured daily urine excretion of adrenaline and noradrenaline in both hyperthyroid and normal subjects. A large increase in adrenaline excretion was found in hyperthyroidism, but there was no change in noradrenaline excretion. There was a correlation between adrenaline excretion and severity of hyperthyroidism, as measured by output of labeled hormone following a tracer dose of $I^{131}$. Possible mechanisms for these changes and relationship to insulin hypoglycemia are discussed. C.A.R.

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*Easley, Albert S.* (Chattanooga, Tenn.): THE USE OF THE ORAL SULFONYLUREAS IN DIABETES MELLITUS. J. Tennessee M. A. *51*:417-22, October 1958.

A review of the history, theory, and technic in use of oral sulfonylurea compounds. Indications and contraindications are explained. A.S.R.

*Ellenberg, Max* (Dept. of Med., Mt. Sinai Hosp., New York, N. Y.): DIABETIC NEUROPATHY PRESENTING AS THE INITIAL CLINICAL MANIFESTATION OF DIABETES. Ann. Int. Med. *49*:620-31, September 1958.

This is a detailed report of seven diabetic patients with diabetic neuropathy as the presenting clinical syndrome. This antedated the clinical onset of diabetes by a period of time or, in some, the diagnosis of diabetes was made only by a glucose tolerance test. The need for increased suspicion of diabetes in such cases is emphasized both for its diagnostic and therapeutic importance. It was also inferred that diabetic neuropathy could exist prior to metabolic manifestation of diabetes. However, this conclusion was not supported by adequate evidence (such as glucose tolerance test) that diabetes did not exist prior to the onset of the neuropathy. S.B.B.

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*Fleming, J. Will, Jr.* (Moberly, Mo.): ORAL TREATMENT OF DIABETES WITH TOLBUTAMIDE. Missouri Med. *55*:1091-94, October 1958.

This paper gives a brief review of the history of oral hypoglycemic agents and the criteria used for instituting tolbutamide therapy, and also gives the author's method in substituting tolbutamide for insulin. He lists the thirteen patients, all older than forty years of age, in whom he has tried tolbutamide. In ten patients, the drug has been useful over an average span of thirteen months. R.L.J.

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*Fredrickson, Donald S.; and Gordon, Robert S., Jr.* (Sect. on Metab., Lab. of Cell Physiol., Nat. Heart Inst., Nat. Inst. of Health, Bethesda, Md.): THE METABOLISM OF ALBUMIN-BOUND $C^{14}$-LABELED UNESTERIFIED FATTY ACIDS IN NORMAL HUMAN SUBJECTS. J. Clin. Invest. *37*:1504-15, November 1958.

The metabolism of plasma unesterified fatty acids (UFA) was studied in twenty-one normal human subjects. Albumin-bound carboxyl-labeled palmitic, oleic and linoleic acid were injected and the removal from plasma and its excretion as expired $CO_2$ were measured. Carbohydrate feeding decreased both the flux and the oxidation of UFA.

The analysis of the data confirmed the rapid distribution of UFA into cellular compartments, far more quickly than its albumin "carrier," as well as rapid oxidation at those sites. A general scheme was outlined for normal UFA metabolism as a background for later comparison with that in abnormal metabolic states. S.B.B.

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*Goldner, Martin G.* (Jewish Chronic Disease Hosp., Brooklyn, N. Y.): ORAL HYPOGLYCEMIC AGENTS PAST AND PRESENT: OTHER THAN SULFONYLUREA COMPOUNDS. A.M.A. Arch. Int. Med. *102*:830-40, November 1958.

Of the long series of the most diverse substances for which hypoglycemic activities have been claimed in the past, only a few appear to have any significant effect. None of these acts like insulin, and none can therefore be considered as an insulin substitute. Some appear to stimulate insulin production or secretion; others, to enhance its peripheral activity, probably by inhibiting its enzymatic destruction; still others seem to act on the cellular metabolism in the periphery. Toxicity or harmful side effects will preclude the clinical application of some of them. But clinical evaluation, wherever possible, is warranted in spite of the availability and proven efficacy of insulin.

Since the etiology of diabetes mellitus is unknown and since it appears likely that peripheral factors may play a role in it as well as the primary deficiency of the insulin-producing

islet-cell system, such evaluation may lead to a rational and possibly causal therapy for at least some type of the disease.

As yet, the arylsulfonylureas are the only substances of this group which have been proven to be useful in the management of one well-defined sector of diabetes mellitus. The presently available evidence raises similar hopes for the hypoglycemic biguanides. B.F.K.

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*Granville-Grossman, K. L.; Crawfurd, Susanne; and Bloom, Arnold.* (Whittington Hospital, London, N. 19, England): CHLORPROPAMIDE. Lancet *2*:803, Oct. 11, 1958.

The authors report their experience with forty-one diabetic patients treated with chlorpropamide. The incidence of toxic side effects was low. C.A.R.

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*Grayson, J.; and Kinnear, T.* (Dept. of Physiol., University College, Ibadan, Nigeria): VASCULAR AND METABOLIC RESPONSES OF THE LIVER TO INSULIN. J. Physiol. *144*:52-67, Nov. 10, 1958.

The effect of insulin on liver blood flow, measured by a method of internal calorimetry, was investigated in the rat and the baboon. Marked increases in liver blood flow occurred during insulin hypoglycemia, and could be prevented by the administration of glucose. The blood-flow response was not modified by bilateral adrenalectomy, celiac neurectomy or by the administration of atropine, tetraethylammonium bromide or dibenzyline. Nervous factors and adrenaline release were, therefore, not responsible for the rise in liver blood flow. The rise was absent upon insulin administration following hepatic arterial ligation. The vasodilatation caused by insulin was thus confined to the hepatic arterial radicles. After insulin administration there was a rise in hepatic heat production which corresponded in time with the increase in liver blood flow. It is suggested that the metabolic response of the liver to hypoglycemia caused by insulin may be accompanied by the liberation of a local vasodilator substance in the liver. G.A.W.

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*Havener, William H.* (The Ohio State University, College of Med., Columbus, Ohio): DIABETES: A CASE REPORT ON PREVENTABLE BLINDNESS. Ohio M. J. *54*:1047, August 1958.

The author illustrates by means of a simple and succinct case report the occurrence of gradual loss of vision in a diabetic where definite microaneurysms were seen on thorough ophthalmologic examinations. The cause of visual loss was found to be due to glaucoma and not the diabetic retinopathy. The point is made that by history alone, it may be impossible to differentiate between presbyopia, cataract, macular degeneration, diabetic retinopathy, optic atrophy, or chronic simple glaucoma. Therefore, every patient with visual impairment deserves a careful ophthalmological examination whether he is diabetic or not. G.J.H.

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*Heller, N.; Kalant, N.; and Hoffman, M. M.* (Res. Lab., Jewish Gen. Hosp.; and Depts. of Investigative Med., and Med., McGill University, Montreal, Canada): RELATIONSHIP BETWEEN INSULIN RESPONSIVENESS AND BLOOD GLUCOSE HALF LIFE IN NORMAL AND DIABETIC SUBJECTS. J. Lab. & Clin. Med. *52*:394-401, September 1958.

The authors present a method that permits the measurement of blood glucose half life after glucose loading, before and after the administration of insulin. The former value is taken as an index of glucose tolerance, and the difference between the two as an index of insulin responsiveness. In normal subjects, the half life of intravenously administered glucose ranged from twenty to eighty-five minutes. After the injection of insulin, the half life was 20 ± 6 minutes regardless of its initial value. In diabetic subjects, however, the decrease in glucose tolerance was reflected as increased half life. As this abnormality increased in severity, the ability of exogenous insulin to lower the half life to normal level decreased proportionately. G.J.H.

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*Klimas, John E., Jr.; and Searle, Gordon W.* (Dept. of Physiology, College of Med., State University of Iowa, Iowa City, Ia.): EFFECTS OF TOLBUTAMIDE ON INTESTINAL GLUCOSE ABSORPTION AND BLOOD GLUCOSE LEVELS. Proc. Soc. Exper. Biol. & Med. *98*:901-02, August-September 1958.

The authors demonstrate that in rats glucose absorption from the small intestine was not affected by the presence of tolbutamide in the glucose solution used. G.J.H.

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*Larsson, Yngve* (Kronprinsessan Lovisa's Barnsjukhus, Stockholm, Sweden): TOLBUTAMIDE TREATMENT OF JUVENILE DIABETES. Quart. Rev. Pediat. *13*:85, May 1958.

The author reported preliminary observations in twenty-four cases of juvenile diabetes treated with tolbutamide, using 2 gm. divided into three or four doses daily in children over ten, and 1 gm. in those younger than ten, with a maintenance dose of 1 gm. and 0.5 gm. respectively. To twelve patients with newly discovered diabetes, insulin and tolbutamide were given simultaneously in a dose sufficient to normalize the blood sugar and eliminate glycosuria; however, this was possible only with a "restricted" diet. According to the author, the insulin was gradually reduced until seven took no insulin and five took very small insulin doses. Another eight patients were given insulin initially, followed by delayed tolbutamide, but only one (over a six-month period) could be kept on tolbutamide alone. Two patients with nothing but abnormal glucose tolerance curves responded to tolbutamide alone which normalized the glucose tolerance. R.L.J.

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*Lewis, J. G.; and Symons, C.* (Royal Free Hospital, London, W.C. 1, England): VASCULAR DISEASE IN A DIABETIC CLINIC. Lancet *2*:985-88, Nov. 8, 1958.

Six hundred and fifty-four diabetics were investigated to determine the incidence of vascular disease and its relationship to age and duration of diabetes, obesity, hypertension, cholesterol levels, and electrocardiographic abnormality. Ninety per cent of the patients were over forty years of age and 73 per cent were female; 42.5 per cent had clinical vascular disease; 59.5 per cent had clinical vascular disease or electrocardiographic abnormalities; including hypertensive patients among those with vascular disease, only 179 (27 per cent) had a "normal" vascular system. The incidence of atherosclerotic heart disease was 19.3 per cent (angina pectoris 11.6 per cent and myocardial infarction 7.8 per cent), of peripheral vascular disease 17 per cent, of retinopathy 21.7 per cent, of nephropathy 4.1 per cent, and of hypertension 36 per cent in men and 56 per cent in women. The majority of patients with vascular disease had more than one complication. Clinical vascular disease was twice as common in hypertensives as in normotensives. Electrocardiographic abnormalities were twice as common in patients with clinical vascular disease as in those without. No relationship was found between clinical vascular disease and cholesterol level. Obesity was slightly more common in patients with clinical vascular disease than in others. Atherosclerotic disease and specific diabetic angiopathy were common in patients over forty years of age at their first attendance. C.A.R.

*Manchester, K. L.; and Young, F. G.* (Dept. of Biochemistry, University of Cambridge, England): THE EFFECT OF INSULIN ON INCORPORATION OF AMINO ACIDS INTO PROTEIN OF NORMAL RAT DIAPHRAGM IN VITRO. Biochem. J. 70:353-58, November 1958.

1. The effect of addition of insulin, glucose and pyruvate on incorporation in vitro into protein of normal rat diaphragm of $C^{14}$ from ($C^{14}$) amino acids and of $S^{35}$ from a ($S^{35}$) amino acid has been studied.

2. Incorporation of radioactivity from the ($C^{14}$) amino acids glycine, leucine, isoleucine, phenylalanine, serine, lysine, arginine, glutamic acid and aspartic acid, each studied separately, and from a mixture of ($C^{14}$) amino acids, was increased by addition of insulin both in the presence and absence of glucose or pyruvate. Neither glucose nor pyruvate greatly affected incorporation in the presence or absence of insulin.

3. Incorporation of $S^{35}$ from ($S^{35}$) methionine sulphoxide was accelerated by addition of insulin both in the presence and absence of glucose or pyruvate. Addition of glucose slightly increased incorporation in the absence of insulin, but neither glucose nor pyruvate affected incorporation in the presence of insulin.

4. Incorporation of $C^{14}$ from alanine was increased in the presence of insulin in the absence of glucose or pyruvate, but was markedly decreased, in the absence of insulin, by addition of pyruvate or, to a less extent, of glucose; in the presence of these substrates insulin did not stimulate incorporation. The possibility is discussed that this behavior of added alanine is attributable to the formation in muscle of alanine from pyruvate or glucose by a process of transamination. C.A.R.

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*Offerijns, F. G. J.; Westerink, D.; and Willebrands, A. F.* (University Dept. of Physiol., Wilhelmina-Gasthuis; Inst. of Human Nutrition, Amsterdam, The Netherlands): THE RELATION OF POTASSIUM DEFICIENCY TO MUSCULAR PARALYSIS BY INSULIN. J. Physiol. *141*:377-84, May 28, 1958.

It is known that patients treated for diabetic coma with large amounts of insulin and replacement fluid may develop generalized paralysis of the extremities and of the respiratory muscles. During the paralysis the potassium level of the serum is extremely low, but when the serum potassium is raised by administration of potassium the paralysis disappears. To investigate these phenomena under controlled conditions, the effect of insulin on the contractions of isolated rat hemidiaphragms following stimulation via the associated phrenic nerve was studied. The diaphragm preparations were obtained from normal rats and from rats fed a potassium-free diet. Neither extracellular nor intracellular potassium deficiency per se caused paralysis of the muscle. On the other hand the addition of insulin (0.2 to 2 units per 100 ml. of suspension fluid) reduced the ability of the potassium-deficient diaphragm to contract. A higher concentration of insulin (40 units per 100 ml. of suspension fluid) caused a relaxation paralysis which was abolished by the addition of 20 mg. of potassium chloride to the suspension fluid. G.A.W.

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*Phillips, Alec* (London, E. 9, England): FALSE GLYCOSURIA. Lancet 2:586, Sept. 13, 1958.

The author reports two instances of positive urine tests with Tes-Tape and Clinistix, both of which failed to reduce Benedict's solution. The bottles in which the specimens were contained had been previously used for hydrogen peroxide, and there was sufficient solution soaked in the screw cap to give a misleading result. The author warns that hydrogen peroxide contamination in urine may mimic glycosuria when these tests are employed. C.A.R.

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*Rafaelsen, Ole J.* (Clin. of Psychiatry and II Clin. of Internal Medicine, Aarhus Univ. Sch. of Med., Copenhagen, Denmark): ACTION OF INSULIN ON ISOLATED RAT SPINAL CORD. Lancet 2:941-43, Nov. 1, 1958.

A technic of isolating rat spinal cord for incubation experiments is described. The results of preliminary experiments were:

1. The basal glucose uptake was 2.0 to 4.5 mg. glucose per gm. spinal cord wet weight per sixty minutes.

2. Insulin in vitro in concentrations from $10^{-1}$ to $10^{-4}$ I.U. per milliliter increased the glucose uptake by 50 per cent.

3. Spinal cord tissue from alloxan diabetic rats had a 25 per cent decreased glucose uptake.

4. Insulin increased the glucose uptake of spinal cord tissue from alloxan diabetic rats. C.A.R.

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*Randle, P. J.; and Smith, G. Howard* (Dept. of Biochemistry, University of Cambridge, England): REGULATION OF GLUCOSE UPTAKE BY MUSCLE 1. THE EFFECTS OF INSULIN, ANAEROBIOSIS AND CELL POISONS ON THE UPTAKE OF GLUCOSE AND RELEASE OF POTASSIUM BY ISOLATED RAT DIAPHRAGM. Biochem. J. 70:490-500, November 1958.

1. Anaerobiosis stimulates the uptake of glucose by isolated rat diaphragm incubated in a medium buffered with bicarbonate. This effect is not observed in media buffered with phosphate or tris (of initial *p*H 7.4). The lack of response to anaerobiosis in the latter media appears to be related to the marked fall in *p*H which occurs during incubation.

2. Under aerobic conditions uptake of glucose by diaphragm in bicarbonate medium is increased by 2:4-dinitrophenol (50 $\mu$M or 0.25 mM), sodium arsenite (mM), sodium arsenate (10 mM), sodium cyanide (mM) and sodium salicylate (5 mM). Sodium azide (mM or 10 mM) and sodium P-hydroxybenzoate (5 mM) do not augment glucose uptake. In a medium buffered with phosphate, 2:4-dinitrophenol (0.25 mM), sodium salicylate (5 mM) and sodium cyanide (mM) fail to stimulate glucose uptake.

3. In bicarbonate medium the uptake of glucose by diaphragm in the presence of 2:4-dinitrophenol, sodium arsenite or sodium salicylate is increased still further by the addition of insulin (0.1 unit/milliliter), though the uptake of glucose achieved under these conditions is less than that with insulin alone. The levels of uptake with insulin under either aerobic or anaerobic conditions were of the same order. In the presence of both insulin and cyanide the uptake of glucose is greater than when either is present by itself.

4. There is a small loss of potassium from diaphragm incubated under aerobic conditions either in bicarbonate or in phosphate medium. The loss of potassium in either medium is very much greater under anaerobic conditions or in the presence of 2:4-dinitrophenol, sodium arsenite, sodium arsenate, sodium cyanide, sodium salicylate or sodium azide at the above concentrations. Insulin effected a slight reduction in the loss of potassium under aerobic conditions. There was no obvious quantitative relation between the loss of potassium from the muscle and the change in glucose uptake.

5. The significance of these results in relation to the mode of uptake of glucose by diaphragm, to the Pasteur effect in

diaphragm and to the mechanism of action of insulin on diaphragm is discussed. C.A.R.

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*Randle, P. J.; and Smith, G. Howard* (Dept. of Biochemistry, University of Cambridge, England): REGULATION OF GLUCOSE UPTAKE BY MUSCLE 2. THE EFFECTS OF INSULIN, ANAEROBIOSIS AND CELL POISONS ON THE PENETRATION OF ISOLATED RAT DIAPHRAGM BY SUGARS. Biochem. J. 70:501-08, November 1958.

1. The accumulation of glucose or xylose in muscle has been studied in vitro with an isolated rat diaphragm preparation in which none of the muscle fibers is cut.

2. The glucose space of this preparation does not exceed the volume of extracellular fluid when diaphragm is incubated aerobically in a bicarbonate-buffered medium. On the other hand, xylose appears to accumulate in about 10 to 15 per cent of the intracellular water under these conditions.

3. Glucose remains extracellular in distribution when diaphragm is incubated anaerobically or in the presence of insulin (0.1 unit/milliliter), sodium cyanide (mM) or sodium salicylate (5 mM), but accumulates within the cells in the presence of sodium arsenite (mM), 2:4-dinitrophenol (0.25 mM) or dinitrophenol (0.25 mM) plus fluoride (10 mM). Each of these factors causes a further accumulation of xylose within the muscle cell.

4. These results provide further support for the views that the entry of glucose into the muscle cell is normally the rate-limiting step in its metabolism; that the glucose entry is restrained by a process dependent on a supply of a substance generated during oxidative phosphorylation; and that insulin may promote glucose uptake by diaphragm by preventing access of this substance to the process regulating glucose entry. C.A.R.

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*Scow, Robert O.; Chernick, Sidney S.; and Smith, Bette-Barron* (Lab. of Nutrition and Endocrinology, Nat. Inst. of Arthritis and Met. Diseases, P.H.S., U.S. Dept. of Health, Bethesda, Md.): KETOSIS IN THE RAT FETUS. Proc. Soc. Exper. Biol. & Med. 98:833-35, August-September 1958.

Blood levels of ketone bodies, glucose, and fats in pregnant rats were altered by fasting and by pancreatectomy. The concentration of ketones and glucose in fetal blood changed with maternal levels, whereas the lipids were independent of the maternal level. Ketone bodies appeared to cross the placenta as rapidly as glucose. G.J.H.

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SEXUAL FUNCTION IN MALE DIABETICS. Brit. M. J. 2:844, Oct. 4, 1958.

Reference is made to a recent study of the reproductive capacity of diabetic men. Impotence was noted in 25 per cent of patients aged thirty to thirty-four, and the incidence rose steadily to almost 75 per cent by the age of sixty. The over-all figures are between two and five times those reported by the Kinsey group in the normal male population. While impotence is particularly common in the early stages of diabetes, potency may return when the disease is adequately controlled. Nevertheless, about 40 per cent of all male diabetics will be found to be permanently impotent, the cause being unknown and the treatment ineffective. The complications of neuropathy have been implicated as a cause. Fertility among diabetic males who are not impotent is said to be normal. There is no evidence that diabetes in the male affects the incidence of prematurity, stillbirths, or congenital malformations in their offspring. C.A.R.

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*Siperstein, Marvin D.; and Fagan, Violet M.* (Dept. of Internal Med., Univ. of Texas, Southwestern Med. School, Dallas, Tex.): STUDIES ON THE RELATIONSHIP BETWEEN GLUCOSE OXIDATION AND INTERMEDIARY METABOLISM. II. THE ROLE OF GLUCOSE OXIDATION ON LIPOGENESIS IN DIABETIC RAT LIVER. J. Clin. Invest. 37:1196-1201, August 1958.

The impaired processes of lipogenesis from $C^{14}$-labeled acetate were studied in liver homogenates of the alloxan diabetic rat. Reinstitution of normal Embden-Meyerhof glycolysis (EM) increased fatty acid synthesis only slightly, whereas restoration to normal of glycolysis by the hexosemonophosphate shunt (HMP) increased lipogenesis to levels well above that of the normal rat. Simultaneous increase in glycolysis by both pathways resulted in a maximal increase in lipogenesis. Cholesterolgenesis was similarly effected. The same effect was produced by the addition to the system of isocitrate and TPN which are capable of furnishing TPNH. It was concluded that the lack of the latter cofactor, supplied by glycolysis by way of the HMP shunt, was the limiting factor in the diabetic defect in lipogenesis. S.B.B.

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*Sokal, Joseph E.; and Sarcione, Edward J.* (Roswell Park Memorial Inst., Buffalo, N. Y.): FAILURE OF BLOOD GLUCOSE LEVELS TO REFLECT HEPATIC GLYCOGENOLYSIS; EXPERIENCES WITH GLUCAGON. Proc. Soc. Exper. Biol. & Med. 98:879-81, August-September 1958.

These authors present impressive data to demonstrate that glucagon is as active a glycogenolytic agent when administered subcutaneously as it is by intraperitoneal or intravenous routes. However, increases in blood glucose may not be observed following subcutaneous administration of glucagon even though liver glycogen is almost completely mobilized. In adrenodemedullated animals, this is the rule. Since the total liver glycogen stored is relatively small compared to the capacity of glucose disposal mechanisms, the changes in the rates of the major glucose disposal reactions can completely mask the anticipated effect of hepatic glycogenolysis on blood sugar levels. Therefore, blood sugar levels do not necessarily reflect the rate of hepatic glycogenolysis. G.J.H.

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*Tyberghein, Jean M.; and Williams, Robert H.* (Dept. of Med., Univ. of Washington Sch. of Med., Seattle, Wash.): ASSAY FOR GLUCAGON IN RABBIT PLASMA. Metabolism 7:635-45, September 1958.

An assay for purified glucagon based on the glycogenolytic effect in surviving rabbit liver slices has been described. Such an assay was shown to be accurate, reproducible and sensitive enough to measure quantities of glucagon in the range of $10^{-6}$ mg. By applying this assay to rabbit plasma extracts, it was possible to demonstrate that they contained a glycogenolytic factor which, for the different aspects studied, was identical to glucagon. It was difficult to establish that the assay values obtained with the extracts reflected the full concentration of glucagon in the plasma. In an attempt to modify this concentration, the following conditions were studied: fasting and treatment with insulin, sulfonylureas, Synthalin A and growth hormone; but no changes were observed. R.W.S.

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*Verner, John V.; and Morrison, Ashton B.* (Depts. of Med. and Path., Duke Univ. Med. Center, Durham, N. C.): ISLET CELL TUMOR AND A SYNDROME OF REFRACTORY WATERY DIARRHEA AND HYPOKALEMIA. Am. J. Med. 25:374-80, September 1958.

This paper draws attention to nine cases of noninsulin-secreting islet cell adenomas which are devoid of beta cells and which

produce an important clinical picture consisting of explosive watery diarrhea, hypokalemic nephropathy with a low fixed specific gravity, hypersecretion of gastric juices, and normal $I^{131}$ Triolein absorption. In some cases, there is recurrent peptic ulceration; in some, pituitary adenoma; and occasional patients with hypercalcemia. R.W.S.

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*Weisenfeld, Shirley* (Dept. of Med. and Isaac Albert Res. Inst., Jewish Chronic Disease Hosp. and Dept. of Med., State University of N. Y. College of Med., Brooklyn, N. Y.): THE EFFECT OF 17 ETHYL 19 NORTESTOSTERONE ON HYPERGLYCEMIC ACTION OF GLUCAGON IN HUMANS. Proc. Soc. Exper. Biol. & Med. 97:764-67, April 1958.

Seventeen ethyl 19 nortestosterone (Nilevar) was administered to sixteen patients and the hyperglycemic response to glucagon was studied. The hyperglycemic response to glucagon was diminished in these patients, but this effect was reversible when Nilevar therapy was stopped. The anabolic material had no apparent effect on the control of prediabetic patients, nor on the twenty-four-hour thyroid iodine$^{131}$ uptakes in eight patients, nor does it seem to be mediated through the adrenal cortex. The possible mechanisms are discussed but are not defined in this article. G.J.H.

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*West, Kelly M.; and McCampbell, Stanley R.* (Dept. of Med., University of Oklahoma School of Med. and Veterans Admin. Hosp., Oklahoma City, Okla.): HYPOGLYCEMIC POTENCY IN MAN OF A NEW SULFONYLUREA DERIVATIVE (CHLORPROPAMIDE). Proc. Soc. Exper. Biol. & Med. 98:724-25, August-September 1958.

The authors administered single oral doses of 1 gm. of chlorpropamide and compared it with the effectiveness of an equivalent dose of tolbutamide in normal subjects. Chlorpropamide produced a significant depression in blood sugar compared to controls at 1, 2, 3, 4, 6, 12, and 18 hr. after the administration of the drug by mouth. Tolbutamide in equivalent doses produced significant lowering of the blood sugar in normal individuals at one, two, three, four, and six hours, but the hypoglycemic effect was not manifest at twelve and eighteen hours. In the light of our present knowledge of the relatively short half life of tolbutamide as compared to the fairly prolonged half life of chlorpropamide, these results are not surprising. G.J.H.

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*Wilkins, Lawson; Jones, Howard W., Jr.; Holman, Gerald H.; and Stempfel, Robert S., Jr.* (Depts. of Pediatrics and Gynecology, The Johns Hopkins University School of Med. & The Harriet Lane Home, Johns Hopkins Hosp., Baltimore, Md.): MASCULINIZATION OF THE FEMALE FETUS ASSOCIATED WITH ADMINISTRATION OF ORAL AND INTRAMUSCULAR PROGESTINS DURING GESTATION: NON-ADRENAL FEMALE PSEUDOHERMAPHRODISM. J. Clin. Endocrinol. *18*:559-85, June 1958.

The writers report twenty-one cases of females born with partial masculinization of the external genitalia, consisting of an enlarged phallus, with or without varying degrees of fusion of the labioscrotal folds. In fifteen of the cases the mother had been treated because of threatened or habitual abortion with an oral progestin, 17-ethinyltestosterone. In two cases, the mother had received intramuscular injections of progesterone. In one case both intramuscular progesterone and oral methyltestosterone had been given. In three cases no steroids were administered during pregnancy. The medication was usually begun before the tenth week of gestation. It is believed that the female fetus is affected in only occasional mothers who receive these steroids, and that in such mothers there may be an abnormality of either the metabolism of progestins or their transmission across the placenta. If diagnosed properly at birth, no treatment except surgical correction of the abnormalities of the external genitalia is required. Normal female development is certain. The abstractor felt that it was pertinent to include this abstract as many physicians are using these compounds in the treatment of pregnant diabetics. Therefore, they should be alert to the occurrence of this syndrome in some patients. G.J.H.

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*Winter, M.* (*Szeged, Hungary*): DINITROPHENOL AND DIABETES MELLITUS. Brit. M. J. 2:976, Oct. 18, 1958.

The author refers to his own and other experiments on the effect of dinitrophenol in intact and pancreatectomized dogs. DNP exerts a remarkable hyperglycemic effect on intact dogs, while a small decrease of the blood sugar level can be observed in pancreatectomized animals. The increase in oxygen consumption, hind-leg blood flow, and blood lactic acid level was the same in the two groups of animals, but the serum inorganic phosphorus level showed a much higher increase in the diabetic dogs. Also noted was a diminished glucose uptake by the muscles of the diabetic dogs in vivo; the blood sugar nevertheless decreased. The significance of these results is briefly discussed. C.A.R.

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*Wiseman, M. H.; Kalant, N.; and Hoffman, M. M.* (Res. Lab., Jewish Gen. Hosp. & Dept. of Investigative Med.; McGill University, Montreal, Canada): TRYPTOPHAN METABOLISM IN NORMAL AND DIABETIC SUBJECTS. J. Lab. & Clin. Med. 52:27-33, July 1958.

The authors present technics for the evaluation of tryptophan metabolism by measuring the urinary excretion of various tryptophan metabolites following the ingestion of a loading dose of tryptophan. The test was performed on elderly diabetic subjects who were found to excrete a subnormal proportion of the administered load in the form of tryptophan kynurenine, anthranilic acid, xanthurenic acid, but normal amounts of 5-hydroxyindoleacetic. The loading dose of tryptophan is stated to have caused a minor decrease in blood sugar concentration in the diabetic subjects, but this is of questionable statistical significance. G.J.H.

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*Zetterström, R.; Strindberg, B.; and Arnhold, R. G.* (Pediatric Clinic, Karolinska Sjukhuset, Stockholm, Sweden): HYPERBILIRUBINEMIA AND ABO HEMOLYTIC DISEASE IN NEWBORN INFANTS OF DIABETIC MOTHERS. Acta Pædiat. 47:238-50, May 1958.

In twenty-nine consecutive cases of newborn infants of diabetic mothers, the serum bilirubin levels during the neonatal period have been studied. These infants are predisposed to the development of hyperbilirubinemia. The average bilirubin levels of this group exceeded those of normal newborns. In the majority, hemolytic disease or other causes of jaundice could be excluded. Hyperbilirubinemia was considered to be due to functional immaturity of the liver. The diagnosis of ABO hemolytic disease was made in five of the series. The high frequency of this complication seems to show that development of this disorder is favored in diabetic pregnancies. R.L.J.

# *ORGANIZATION SECTION*

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## NINETEENTH ANNUAL MEETING JUNE 6-7, 1959

The Nineteenth Annual Meeting of the American Diabetes Association will be held in Atlantic City, New Jersey, just prior to the annual session of the American Medical Association. The ADA's first Scientific Session will be presented on Saturday morning, June 6, and the second and third Sessions on Sunday morning and afternoon, June 7. Among highlights of the Sunday Sessions will be a panel on the oral hypoglycemic agents, details of which will be published in the May-June issue of the Journal. Please note that no Scientific Session will be held on Saturday afternoon, to enable members to attend Endocrine Society meetings in which they may be interested.

Although a joint meeting per se will not be held with The Endocrine Society, arrangements have been made so that each organization will recognize the registration badges of the other. Endocrine Society members, therefore, may attend the Scientific Sessions of the American Diabetes Association and ADA members may attend, without charge, sessions on diabetes of The Endocrine Society.

The program of The Endocrine Society on Saturday afternoon, June 6, will be on insulin and diabetes. The ADA Assembly of Delegates, which includes the membership of the Board of State Governors, is scheduled to meet the same afternoon.

Members and their wives (or husbands) and friends are invited to attend both the Annual Banquet and Social Hour Saturday evening, June 6. A reservation form for the Banquet will be mailed to all members in advance of the meeting.

A special hotel reservation card was sent in November to all Association members. Those who have not yet filled it out are urged to do so immediately and send it directly to Chalfonte-Haddon Hall, the headquarters hotel in Atlantic City.

Members planning to attend the ADA Annual Meeting and wishing to stay over at the same hotel for the AMA sessions June 8-12, must make separate reservations for the AMA sessions through the Atlantic City Convention Bureau. A reservation form for this purpose appeared on page 224 of the Feb. 14, 1959, issue of *The Journal of the American Medical Association.* Please indicate on this form or by letter that you would like to continue your previous ADA reservation at Chalfonte-Haddon Hall for the AMA sessions.

DIABETES will publish a list of papers to be given at the Scientific Sessions; and the program itself, including abstracts of all papers presented or read by title, will be sent to all members without charge before the meeting.

Members and nonmembers may secure additional copies of the final Program at a cost of $1.00 each by requesting them from the American Diabetes Association, 1 East 45th St., New York 17, N. Y. The program also will be available at the Registration Desk in Atlantic City for those who wish to purchase one at the time of the Meeting.

## EIGHTH POSTGRADUATE COURSE

The American Diabetes Association will hold its Eighth Postgraduate Course Jan. 20, 21 and 22, 1960, at the Hotel Ambassador in Los Angeles, California. Registration fees will be $40 for Association members and $75 for nonmembers. All inquiries pertaining to the Course should be directed to the national office, 1 E. 45th St., New York 17, N. Y.

Thaddeus S. Danowski, M.D., Chairman of the ADA Committee on Professional Education, will be Course Director. Roy F. Perkins, M.D., the Association's Governor for Southern California, will be chairman of the local committee.

## RESEARCH FELLOWSHIPS AWARDED

The Committee on Research and Fellowships of the American Diabetes Association announces the award, for the 1959-1960 academic year (July 1, 1959—June 30, 1960), of the following Research Fellowships:

*Adler Foundation Research Fellowship.* David Norman, Ph.D., of Shrewsbury, Massachusetts, will continue his work under the three-year Adler Foundation Research Fellowship. Dr. Norman is studying the action of insulin on the rat diaphragm under a variety of conditions, which include the effect of other hormones on the penetration of sugars into this tissue. The research is being carried out under the cooperative direction of Dr. William A. Hiestand of Purdue University and Dr. Oscar Hechter of the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts.

Bruce M. Carruthers, M.D., Philadelphia, Pennsylvania, to study the relation between a defect in glucose-6-phosphate dehydrogenase activity of the red cells which occurs in certain persons and their systemic response to tests of carbohydrate metabolism and insulin sensitivity. Dr. Carruthers will work with Francis C. Wood, M.D., Professor of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

June W. Pryor, M.D., Boston, Massachusetts, to study the relation of glucagon to protein metabolism by in vitro methods. Dr. Pryor will work with George W. Thorn, M.D., and Albert E. Renold, M.D., at the Baker Clinic Research Laboratory, Peter Bent Brigham Hospital, Boston, Massachusetts.

Jurgen Steinke, M.D., Boston, Massachusetts, for the study of blood insulin levels in diabetic patients, in the Baker Clinic Research Laboratory, Peter Bent Brigham Hospital, Boston, Massachusetts, with George W. Thorn, M.D., and Albert E. Renold, M.D.

The Committee on Research and Fellowships plans to award at least one Fellowship for the academic year 1960-1961. The deadline for applications is Nov. 15, 1959. Requests for application forms and other inquiries should be addressed to Mr. J. Richard Connelly, Executive Director, who will forward the information to the Committee.

## ADA AMENDS BYLAWS

The Council of the American Diabetes Association on Jan. 24-25, 1959, adopted the following amendments to the Bylaws: *Addition of a Paragraph 4 to Article VI (Committees), Section 3 (Standing Committees) as follows*:

4. The Committee on Investment to consist of the Treasurer, Chairman of the Committee on Finance and three other members selected by the President with the approval of the Executive Committee. The latter three members to be selected from members of the Council and/or past Presidents and past Treasurers of the Association in such manner that the term of one such member shall expire each year and his successor be appointed as aforesaid for a term of three years.

   The Committee shall be responsible for carrying out the investment program of the Association and for the investment and reinvestment of funds as and in the manner designated by the Council. The Treasurer or such other officer or agent as may be designated by the Council, is authorized to purchase and sell stocks, bonds and other securities and to sign receipts, bills of sale and such other instruments as may be proper in the execution of such duties; provided, however, that no such sale, investment or reinvestment of any funds or property shall be made unless and until such sale, investment or reinvestment shall be approved by the majority of the members of the Committee on Investment. It shall be proper for the Committee to act upon the affirmative written vote of a majority of the members thereof.

   The Committee on Investment shall be responsible to the Council and shall report to the Executive Committee and to the Council at each regularly constituted meeting of each of these bodies.

*Amendment of final sentence of Article VI, Section 3, Paragraph 1, to read*:

This Committee shall consider and propose methods of obtaining funds and shall consult with the Treasurer on matters concerning the general finances of the Association.

## APRIL 1 DEADLINE FOR MEDICAL STUDENT-INTERN ESSAY CONTEST

April 1, 1959, is the deadline for submission of entries in the 1958-1959 (seventh) Student-Intern Essay Contest for which all medical students, interns and physicians within two years after their graduation from medical school are eligible.

The author(s) of the best paper reporting original work, whether laboratory investigation or clinical observation, will be given an award of $250. This prize again has been made possible by the St. Louis Diabetes Association. The author(s) of the best case report or review article will win $100, double the amount of the award in previous years.

Any subject relating to diabetes and basic metabolic problems may be chosen. Criteria for judgment will be the value of the material and the method of presentation.

Original and two copies of manuscripts, typewritten and double-spaced, should be sent to the Committee on Scientific Awards, American Diabetes Association, Inc., 1 East 45th St., New York 17, N. Y.

## FOURTH CONGRESS OF THE INTERNATIONAL DIABETES FEDERATION

The Fourth Congress of the International Diabetes Federation has been scheduled to be held in Geneva, Switzerland, in 1961. The exact date of the meeting and other pertinent information will be published in the Journal as soon as it is received.

## TIMELY ARTICLES IN *ADA FORECAST*

Physicians and others who are concerned with helping diabetic patients and their families will be interested to know that an important article of immediate value to them appeared in the March-April 1959, Volume 12, Number 2, issue of FORECAST. Entitled "Diabetics' Guide to Federal Income Tax Deductions," it offers instructions and suggestions concerning possible deductions diabetics can properly take to get the maximum tax advantage. Physicians may wish to suggest that their diabetic patients read the article for assistance in preparing their federal tax returns.

A list of "Summer Camps for Diabetic Children" is scheduled for the May-June FORECAST, including detailed information about each camp and a map showing geographical locations. The article is of special interest because a number of new camps have been organized and facilities are continually expanding.

Copies of the March-April issue are now available on request, at 35¢ each or six for $2.00. The May-June issue may be obtained after April 15 at the same price, and single copies of reprints of the camp list may be secured without charge.

## DIABETES WEEK, 1959

The week of November 15-21 will be observed as Diabetes Week, following the usual practice of observing official Diabetes Week the week before Thanksgiving. Affiliate Associations and Committees on Diabetes Detection of County and State Medical Societies are urged to note these dates in making plans for development of Public Education and Detection programs.

## NEW MEMBERS

The following were elected as of Feb. 1, 1959, and March 1, 1959:

Active

*California*
Sawtelle, Welles E. — Santa Barbara
*Colorado*
O'Brien, Donough — Denver
*Georgia*
Bloodworth, Frederick A. — Gainesville
Kaufmann, James A. — Atlanta
Rollings, Harry E. — Savannah
Whiteman, Harold W. — Atlanta
*Illinois*
Conroy, William J. — Chicago
*Iowa*
Powell, Robert M. — Mason City
*Kentucky*
Evans, Henry C., Jr. — Harlan
*Maryland*
Blumenfeld, Samuel — Baltimore
*Massachusetts*
Cahill, George F., Jr. — Wellesley
Cristol, David A. — Brighton
Pellegrini, Rinaldo — Boston
*Missouri*
Dames, Richard J. — St. Louis
Keim, John H. — Cape Girardeau
Peden, Virginia H. — St. Louis
Sharp, Austin R. — St. Louis
*New Jersey*
Most, William — Collingswood
Santangelo, Joseph A. — Newark
*New York*
Dadey, John L. — Syracuse
Ginsburg, Selig M. — New York
Scott, Alvin L. — Buffalo
Smith, Asa J. — Oneida
Stern, Arthur B. — Brooklyn
*Ohio*
Kent, Gerald T. — Cleveland
Kurt, Edward J. — Toledo
Mackenzie, Malcolm S. — Cleveland
*Oregon*
Partridge, John W. — Portland
*Pennsylvania*
Goto, Yoshio — Philadelphia
*South Dakota*
Whitney, Nathaniel R., Jr. — Rapid City
*Texas*
Farley, James A. — Fort Worth
*Virginia*
Grigsby, Bernard C. — Bristol
*Wisconsin*
Roth, Donald A. — Milwaukee

Other Countries

*Canada*
Leith, Wilfred — Montreal
*Venezuela*
Ruiz, Miguel R. — Caracas

Associate

*New York*
McFarland, Mr. Henry J. — Albany

## *NEWS OF AFFILIATE ASSOCIATIONS*

The NEW YORK DIABETES ASSOCIATION (Clinical Society) held an open meeting Thursday, Feb. 26, 1959, at the New York Academy of Medicine. W. A. Cochrane, M.D., Associate Professor of Pediatrics, Dalhousie University, School of Medicine, Halifax, Nova Scotia, spoke on "The Relationship of Amino Acids to Idiopathic Hypoglycemia," with discussion by Henry Aranow, Jr., M.D., Assistant Professor of Clinical Medicine, Columbia University College of Physicians and Surgeons. Paul Wermer, M.D., Associate in Medicine, Columbia University College of Physicians and Surgeons, spoke on "Multiple Endocrine Adenomatosis with Hypoglycemia," with discussion by Robert D. Gittler, M.D., Physician in Charge, Endocrine Laboratories, Beth Israel Hospital.

The OKLAHOMA DIABETES ASSOCIATION held a dinner meeting at the University of Oklahoma School of Medicine in Oklahoma City on Feb. 27, 1959. Fredrick J. Stare, M.D., Harvard University, was speaker. Another meeting is scheduled for May, but arrangements have not yet been completed.

## AN EXTENSION OF LIFETIME SUPPORT

As an extension of their lifetime support, a number of members of the American Diabetes Association have inquired about ways to provide for the continuing welfare of the organization in their wills.

Other members, who may also want to help assure that the essential program of the Association is continued, may wish to give the following form of general bequest to their attorney when they discuss their estate plans with him:

*I hereby give and bequeath to the American Diabetes Association, Inc., a corporation organized under the laws of the State of Ohio, having its principal office at 1 East 45th St., New York 17, N. Y., the sum of ———— dollars.*

The Association's annual income meets only its minimum needs. Each year, its services expand and the costs of its operations increase. The extra financial assistance that bequests represent, therefore, assumes increased importance every year.

Bequests to the Association are exempt from estate taxes. Interested members should ask their attorney about the various tax advantages; and how gifts of real property, as well as cash and securities, can be given. Any questions may be referred to the American Diabetes Association, 1 East 45th St., New York 17, N. Y.

The WASHINGTON DIABETES ASSOCIATION (Clinical Society) will hold its Fifth Annual Symposium on "Endocrine Metabolic Disease" on May 23, 1959, in the Health Sciences Auditorium of the University of Washington. Topics to be discussed are: "Treatment of Nodular Goiter," "Treatment of Pregnancy in Diabetic Women," and "Studies of Fructose Metabolism and the Oral Hypoglycemic Agents." The meeting will be sponsored by the Washington Diabetes Association in connection with the University of Washington Medical School and the State Department of Health.

# *NEWS NOTES*

## AUGUSTA GRADUATE ASSEMBLY

The Augusta Graduate Assembly, sponsored by the Richmond County Medical Society of Augusta, Georgia, will be held April 6 and 7, 1959, at the Bon Air Hotel, Augusta. The Monday afternoon session on April 6, with J. D. Gray, M.D., as chairman, will include: Stefan S. Fajans, M.D., Ann Arbor, "Recent Concepts in the Early Recognition of Diabetes Mellitus"; John Kemble, M.D., "Neuropathies Connected with Diabetes"; Dr. Fajans, "Newer Concepts in the Treatment of Diabetes"; and Roger D. Williams, M.D., Columbus, Ohio, "The Diabetic Patient as a Surgical Risk." Dr. Gray will be moderator of a "Question and Answer Period."

## INTERNATIONAL SOCIETY OF INTERNAL MEDICINE WILL MEET

The Sixth International Congress for Internal Medicine will be held in Basel, Switzerland, Aug. 24-27, 1960. The Congress will be arranged in conjunction with the Swiss Society for Internal Medicine. For further details, apply to the Secretariat of the Sixth International Congress for Internal Medicine, 13, Steinentorstr., Basel.

## PERSONALS

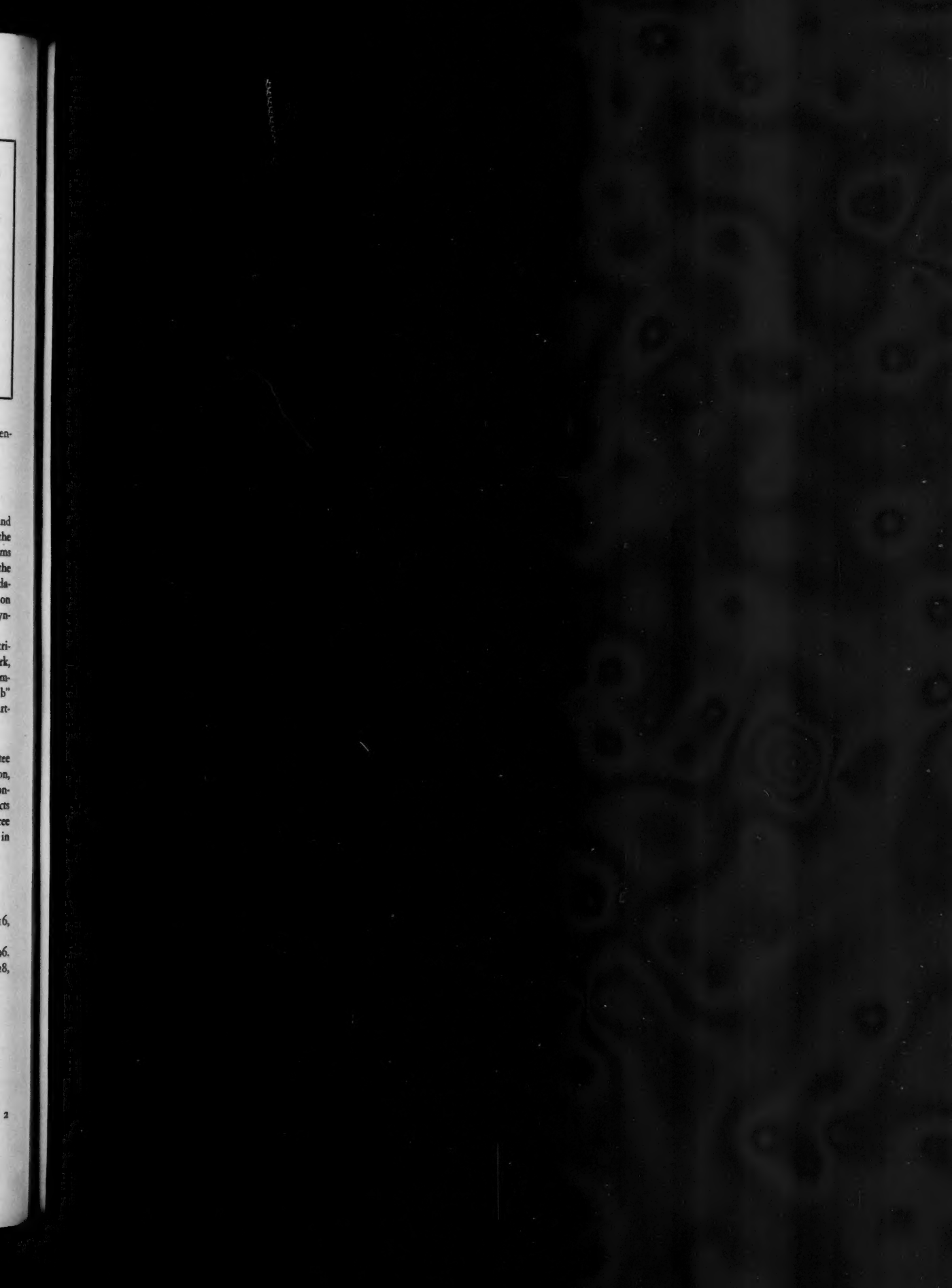
DAVID ADLERSBERG, M.D., The Mount Sinai Hospital and Columbia University, was chairman and moderator of the afternoon session of a symposium on absorption mechanisms and the malabsorption syndrome, held in conjunction with the Fourteenth Annual Meeting of The National Vitamin Foundation in New York City on March 3. Dr. Adlersberg spoke on "Clinical and Pathological Aspects of the Malabsorption Syndrome in Man," and also took part in a panel discussion.

NORMAN JOLLIFFE, M.D., Director of the Bureau of Nutrition of the Department of Health of the City of New York, was principal speaker at the dinner following the annual symposium. The subject of his talk was "The Anti-Coronary Club" (the Diet and Coronary Disease Study Project of the Department of Health of the City of New York).

MARSHALL I. HEWITT, M.D., Chairman of the Committee on Scientific Exhibits of the American Diabetes Association, won the American Medical Writers' Association's annual contest in nonmedical writing for his paper entitled "Side Effects of Exhibiting Scientifically." Dr. Hewitt was selected from three finalists who read their papers at the A.M.W.A. Banquet in Chicago Sept. 26, 1958.

## NECROLOGY

LOUIS E. AUDET, Williamsport, Pennsylvania, born Sept. 16, 1904.

WILLIAM J. BRYAN, JR., Tulsa, Oklahoma, born Feb. 1, 1896.

MATTHEW TAUBENHAUS, Chicago, Illinois, born Nov. 28, 1903.